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<b>(21) International Application Number:</b> PCT/DK93/00395 <b>(22) International Filing Date:</b> 1 December 1993 (01.12.93)  <b>(30) Priority Data:</b> <table border="0" style="width: 100%;"><tr><td style="width: 30%;">1441/92</td><td style="width: 40%;">1 December 1992 (01.12.92)</td><td style="width: 30%;">DK</td></tr><tr><td>772/93</td><td>29 June 1993 (29.06.93)</td><td>DK</td></tr><tr><td>1197/93</td><td>25 October 1993 (25.10.93)</td><td>DK</td></tr><tr><td>1200/93</td><td>26 October 1993 (26.10.93)</td><td>DK</td></tr></table> <b>(71) Applicant (for all designated States except US):</b> NOVO NORDISK [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SCHNEIDER, Palle [DK/DK]; Rydtoften 43, DK-2750 Ballerup (DK). EBDRUP, Søren [DK/DK]; Willemoesgade 89, st. t.h., DK-2100 Copenhagen Ø (DK).  <b>(74) Common Representative:</b> NOVO NORDISK A/S; Patent Department, Novo Allé, DK-2880 Bagsvaerd (DK).		1441/92	1 December 1992 (01.12.92)	DK	772/93	29 June 1993 (29.06.93)	DK	1197/93	25 October 1993 (25.10.93)	DK	1200/93	26 October 1993 (26.10.93)	DK	<b>(81) Designated States:</b> BR, CA, FI, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
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<b>(54) Title:</b> ENHANCEMENT OF ENZYME REACTIONS  <b>(57) Abstract</b>  This invention relates to activation of enzymes. More specifically, the invention relates to agents capable of enhancing the activity of peroxidases or peroxidase acting compounds. The invention also relates to methods of oxidizing a substrate with a source of hydrogen peroxide in the presence of a peroxidase enzyme or a peroxidase acting compound, and an enhancing agent. More specifically, the invention relates to a method of bleaching of dye in solutions, to a method of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, to a method of bleaching of lignin-containing material, in particular bleaching of pulp for paper production, to a method of treatment of waste water from pulp manufacturing, and to a method of enzymatic polymerization and/or modification of lignin or lignin containing material.														

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## ENHANCEMENT OF ENZYME REACTIONS

### TECHNICAL FIELD

This invention relates to activation of enzymes. More specifically, the invention relates to agents capable of enhancing the activity of peroxidases or peroxidase acting compounds.

The invention also relates to methods of oxidizing a substrate with a source of hydrogen peroxide in the presence of a peroxidase enzyme or a peroxidase acting compound, and an enhancing agent. More specifically, the invention relates to a method of bleaching of dye in solutions, to a method of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, to a method of bleaching lignin-containing material, in particular bleaching of pulp for paper production, to a method of treatment of waste water from pulp manufacturing, and to a method of enzymatic polymerization and/or modification of lignin or lignin containing material.

### BACKGROUND ART

Peroxidases (E.C. 1.11.1.7) are enzymes that catalyse the oxidation of a substrate (an electron or hydrogen donor) with hydrogen peroxide. Such enzymes are known from microbial, plant and animal origins, e.g. peroxidase from Coprinus cinereus (cf. e.g. EP 179,486). They are typically hemoproteins, i.e. they contain a heme as a prosthetic group.

Use of peroxidase together with hydrogen peroxide or a hydrogen peroxide precursor has been suggested e.g. in bleaching of pulp for paper production, in treatment of waste water from pulp production, for improved bleaching in laundry detergents, for dye transfer inhibition during laundering, and for lignin modification, e.g. in particle board production.

The compound 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate), ABTS, supplied by Boehringer Mannheim, is a

chromogenic substrate, and a common peroxidase and phenol oxidase assay agent. These enzymes catalyse the oxidation of ABTS by hydrogen peroxide and dioxygen, respectively, producing a greenish-blue colour, which process may be monitored photo-  
5 metrically.

ABTS has been found to form a stable radical cation when oxidized by a laccase enzyme (polyphenol oxidase, EC 1.10.3.2), and has been proposed to act as a redox mediator for oxidation of non-phenolic lignin model compounds [Bourbonnais  
10 R. Paice M G; FEBS Lett (1990) 267 99-102].

Studies on demethylation and delignification of kraft pulp by a laccase enzyme in the presence of ABTS showed that the extent of partial demethylation by laccase was increased in the presence of ABTS [Bourbonnais, R. and Paice,  
15 M.G; Appl. Microbiol. Biotechnol. (1992) 36 823-827].

Certain oxidizable substrates e.g. metal ions and phenolic compounds such as 7-hydroxycoumarin (7HcM), vanillin (VAN), and p-hydroxybenzenesulfonate (pHBS), have been described as accelerators or enhancers, able to enhance bleaching  
20 reactions (cf. e.g. WO 92/18683, WO 92/18687, and Kato M and Shimizu S, Plant Cell Physiol. 1985 26 (7), pp. 1291-1301 (cf. Table 1 in particular), or Saunders B C, et al., Peroxidase, London, 1964, p. 141 ff).

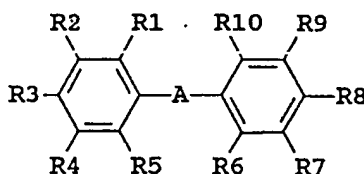
#### SUMMARY OF THE INVENTION

25 It has now surprisingly been found that organic chemical compounds consisting of at least two aromatic rings, of which aromatic rings at least one ring is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur, and which aromatic rings may furthermore be fused  
30 rings, are capable of enhancing the activity of peroxidases and peroxidase acting compounds.

Accordingly, in its first aspect, the invention provides an agent for enhancing the activity of peroxidases or peroxidase acting compounds, which agent is an organic chemical  
35 compound consisting of at least two aromatic rings, of which

aromatic rings at least one ring is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

In a more specific aspect, the invention provides an agent for enhancing the activity of peroxidases or peroxidase acting compounds, which agent is an organic chemical compound of the general formula I:



in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=N-$   
 10  $)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{13}-NR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-$   
 $)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ ,  $(-N=N-)$ , in which groups  
 n represents an integer of from 1 to 6; or A represents carbon,  
 carbonyl, nitrogen, sulfur, oxygen, selenium, or phosphor,  
 which carbon, phosphor and nitrogen may be unsubstituted or  
 15 substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  
 $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, indepen-  
 dently represents any of the following radicals: hydrogen,  
 halogen, hydroxy, formyl, carboxy and esters and salts hereof,  
 20 carbamoyl, sulfo and esters and salts hereof, sulfamoyl,  
 phospho, phosphono, phosphonooxy, sulfandiyl, nitro, amino,  
 phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ -  
 $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may  
 furthermore be unsubstituted or substituted once or twice with  
 25 a substituent group  $R^{14}$ ; and which phenyl may furthermore be  
 unsubstituted or substituted with one or more substituent  
 groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -  
 alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or un-  
 saturated, branched or unbranched, and may furthermore be  
 30 unsubstituted or substituted with one or more substituent  
 groups  $R^{14}$ ;

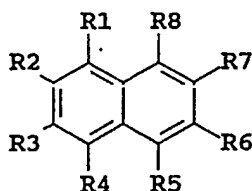
which substituent group  $R^{14}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperaziny, pyrrolidino,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula  $R^5$  and  $R^6$  may together form a group -B-, in which B represents a single bond, the group  $(-CH=CH-)_n$ , or the group  $(-CH=N-)_n$ , in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{14}$  as defined above;

or in which general formula two of the substituent groups  $R^1$ - $R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to 4, and in which groups  $R^{11}$  is a substituent group as defined above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when n in the above formula being 1, may together form a group -D-, in which D represents the groups:  $(-CHR^{11}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=CR^{15}-)$ ,  $(-CR^{11}=N-)$ ,  $(-N=CR^{11}-)$ ,  $(-O-)$ ,  $(>C=O)$  or  $(-S-)$ , and in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

In another specific aspect, the invention provides an agent for enhancing the activity of peroxidases or peroxidase acting compounds, which agent is an organic chemical compound of the general formula II:



5 in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ -  
 10  $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  
 15  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the  
 20 following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with  
 25 hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts

hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>3</sub>-alkyl, and C<sub>1</sub>-C<sub>3</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any  
5 of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B presents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

In another aspect, the invention provides a method  
15 for oxidizing a substrate with a peroxidase or a peroxidase acting compound, in the presence of a source of hydrogen peroxide, in the presence of an enhancing agent of the invention.

In a more specific aspect, the invention provides a  
20 method for bleaching dye in solutions by treatment with a peroxidase enzyme or a peroxidase acting compound in the presence of a source of hydrogen peroxide in the presence of an enhancing agent of the invention.

In another specific aspect, the invention provides  
25 a method of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the method comprising treatment of the wash liquor with a peroxidase or peroxidase acting compound in the presence of a source of hydrogen peroxide and in the  
30 presence of an enhancing agent of the invention.

In another aspect, the invention provides a method of bleaching lignin-containing material, in particular bleaching of pulp for paper production, the method comprising treatment of the lignin or lignin containing material with a  
35 peroxidase or a peroxidase acting compound in the presence of a source of hydrogen peroxide and in the presence of an enhancing agent of the invention.



In a further aspect, the invention provides a method of enzymatic polymerization and/or modification of lignin or lignin containing material, the method comprising treatment of the lignin or lignin containing material with a peroxidase or a peroxidase acting compound in the presence of a source of hydrogen peroxide and in the presence of an enhancing agent of the invention.

In a yet further aspect, the invention provides a method of treatment of waste water, in particular waste water from pharmaceutical or chemical industry, e.g. waste water from dye manufacturing, from textile industry, or from pulp manufacturing, the method comprising treatment of the waste water with a peroxidase or a peroxidase acting compound in the presence of a source of hydrogen peroxide and in the presence of an enhancing agent of the invention.

In a particular aspect, the invention provides a detergent additive capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent additive comprising an enzyme exhibiting peroxidase activity or a peroxidase acting compound, a source of hydrogen peroxide and an enhancing agent of the invention.

In other aspects, the invention provides detergent additives and detergent compositions capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent composition comprising an enzyme exhibiting peroxidase activity or a peroxidase acting compound, a source of hydrogen peroxide, and an enhancing agent of the invention.

30

#### BRIEF DESCRIPTION OF DRAWINGS

The present invention is further illustrated by reference to the accompanying drawings, in which:

Fig. 1 shows the specific formulas of some enhancing agents of the invention (I) 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS)); (II) 6-hydroxy-2-naphtoic acid;

(III) 6-bromo-2-naphtol; (IV) 7-methoxy-2-naphtol; (V) 7-amino-2-naphthalene sulfonic acid; (VI) 5-amino-2-naphthalene sulfonic acid; (VII) 1,5-diaminonaphthalene; (VIII) 7-hydroxy-1,2-naphthimidazole; (IX) 10-methylphenothiazine; (X) 10-pheno-  
 5 thiazine-propionic acid; (XI) N-hydroxysuccinimide-10-pheno-  
 thiazine-propionate; (XII) benzidine; (XIII) 3,3'-dimethylben-  
 zidine; (XIV) 3,3'-dimethoxybenzidine; (XV) 3,3',5,5'-tetrame-  
 thylbenzidine; (XVI) 4'-hydroxy-4-biphenylcarboxylic acid;  
 (XVII) 4-amino-4'-methoxystilbene; (XVIII) 4,4'-diaminostil-  
 10 bene-2,2'-disulfonic acid; (XIX) 4,4'-diaminodiphenylamine;  
 (XX) 2,7-diaminofluorene; (XXI) 4,4'-dihydroxy-biphenylene;  
 (XXII) triphenylamine); (XXIII) 10-ethyl-4-phenothiazinecar-  
 boxylic acid; (XXIV) 10-ethylphenothiazine; (XXV) 10-propyl-  
 phenothiazine; (XXVI) 10-isopropylphenothiazine; (XXVII)  
 15 methyl-10-phenothiazinepropionate; (XXVIII) 10-phenylphenothia-  
 zine; (XXIX) 10-allylphenothiazine; (XXX) 2-chloro-10-methyl-  
 phenothiazine; (XXXI) 10-(3-(4-methyl-1-piperazinyl)propyl)phe-  
 nothiazine; (XXXII) 10-(2-pyrrolidinoethyl)phenothiazine;  
 (XXXIII) chlorpromazine; (XXXIV) 10-methylphenoxazine; (XXXVI)  
 20 6-amino-3-methyl-2-benzothiazolinone azine with 3-methyl-2-  
 benzothiazolinone; (XXXVII) iminostilbene; (XXXVIII) 2-(p-  
 aminophenyl)-6-methylbenzothiazole-7-sulfonic acid; (XXXIX) N-  
 benzylidene-4-biphenylamine; (XL) 4,4'-diaminodiphenylamine-  
 sulfate; (XLI) 5-amino-2-naphthalenesulfonic acid; (XLII) 1,6-  
 25 dibromo-2-naphtol; (XLIII) 7-methoxy-2-naphtol; (XLIV) 4,4'-  
 dihydroxybenzophenone; (XLV) N-(4-(dimethylamino)benzylidene)-  
 p-anisidine; (XLVI) 3-methyl-2-benzothiazolinone(4-(dimethyl-  
 amino)benzylidene)hydrazone; (XLVII) 2-acethyl-10-methyl-  
 phenothiazine.

30 Fig. 2 shows a comparison of an enhancing agent of  
 the invention (ABTS) and PHBS, applied to bleaching of Methyl  
 Orange by a Coprinus cinereus peroxidase (1: PHBS, 20  $\mu\text{M}$   $\text{H}_2\text{O}_2$ ;  
 2: PHBS, 200  $\mu\text{M}$   $\text{H}_2\text{O}_2$ ; 3: ABTS, 20  $\mu\text{M}$   $\text{H}_2\text{O}_2$ ; 4: ABTS, 200  $\mu\text{M}$   $\text{H}_2\text{O}_2$ );

Fig. 3 shows accelerated bleaching of Methyl Orange  
 35 by a Coprinus cinereus peroxidase in the presence of varying  
 concentrations of an enhancing agent of the invention (ABTS)  
 (1: 0  $\mu\text{M}$  ABTS; 2: 1  $\mu\text{M}$  ABTS; 3: 5  $\mu\text{M}$  ABTS; and 4: 10  $\mu\text{M}$  ABTS);

Fig. 4 shows a comparison of the initial bleaching rates during bleaching of Direct Blue 1 (DB1) at pH 10.5 (□ ABTS, 1 nM peroxidase; ♦ VAN, 100 nM peroxidase; ■ 7HCm, 100 nM peroxidase; ▲ pHBS, 100 nM peroxidase); and

5 Fig. 5 shows a comparison of the initial bleaching rates during bleaching of DB1 at pH 8.8 (and pH 10.5) (□ ABTS pH 8.8; ♦ VAN pH 8.8; ■ 7HCm pH 8.8; ◇ ABTS pH 10.5; and ▲ pHBS pH 8.8).

Fig. 6 shows the initial bleaching of DB1 at pH 10.5  
10 under the following conditions: 100  $\mu$ M  $H_2O_2$ ; 25 mM Borate; 0.5 PODU/ml Myxococcus virescens peroxidase; 0  $\mu$ M 10-propionic acid phenothiazine (□)/ 10  $\mu$ M 10-propionic acid phenothiazine (♦), respectively; room temperature.

Determination of peroxidase activity: 1 peroxidase unit  
15 (PODU) is the amount of enzyme that catalyzes the conversion of 1  $\mu$ mol hydrogen peroxide per minute at the following analytical conditions: 0.88 mM hydrogen peroxide, 1.67 mM 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate), 0.1 M phosphate buffer, pH 7.0, incubated at 30°C, photometrically followed at 418 nm.

20

#### DETAILED DISCLOSURE OF THE INVENTION

##### The Enhancing Agent

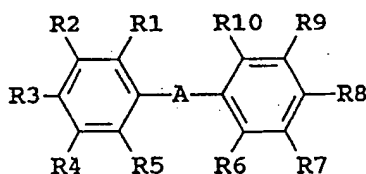
The present invention relates to the use of chemical compounds for enhancing the activity of peroxidase enzymes or peroxidase acting compounds. Accordingly, the invention  
25 provides an agent capable of enhancing the effect of a peroxidase enzyme or a peroxidase acting compound, in the following termed enhancing agent.

Contrary to the enhancers known and previously described, the enhancing agents of this invention are capable  
30 of acting at alkaline conditions, i.e. at pH above 8. This feature allows the enhancers of the invention to be implemented into various industrial processes.

The enhancing agent of the invention is an organic chemical compound consisting of at least two aromatic rings, of

which aromatic rings at least one ring is substituted with one or more nitrogen, oxygen, and/or sulfur atoms, and which aromatic rings may furthermore be fused rings.

In a more preferred embodiment, the enhancing agent of the invention is an organic chemical compound of the general formula I:



in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-NR^{11}-)_n$ ,  $(-CR^{11}=N-N=CR^{13}-NR^{15}-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{13}-NR^{15}-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{13}-)_n$ ,  $(-CR^{12}=N-NR^{11}-)_n$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)_n$ ,  $(-N=N-)_n$ , in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or phosphor, which carbon, phosphor and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy, sulfandiyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or un-

saturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperaziny, pyrrolidino,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula  $R^5$  and  $R^6$  may together form a group -B-, in which B represents a single bond, the group  $(-CH=CH-)_n$ , or the group  $(-CH=N-)_n$ , in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{14}$  as defined above;

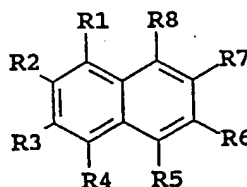
or in which general formula two of the substituent groups  $R^1$ - $R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to 4, and in which groups  $R^{11}$  is a substituent group as defined above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when n in the above formula being 1, may together form a group -D-, in which D represents the groups:  $(-CHR^{11}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=CR^{15}-)$ ,  $(-CR^{11}=N-)$ ,  $(-N=CR^{11}-)$ ,  $(-O-)$ ,  $(>C=O)$  or  $(-S-)$ , and

in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

In particular embodiments, the enhancing agent is 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate), 6-amino-3-methyl-2-benzothiazolinone azine with 3-methyl-2-benzothiazolinone, 2-(p-aminophenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethylamino)benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)-hydrazone.

10 In another preferred embodiment, the enhancing agent of the invention is an organic chemical compound of the general formula II:



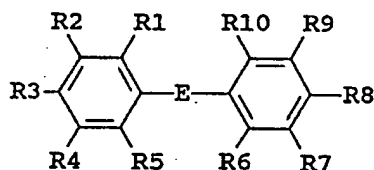
in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or

unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may further-  
 10 more be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranch-  
 15 ed, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent  
 20 groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following groups:  $(-N=N-)_n$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ ,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

25 In a more specific embodiment, the enhancing agent of the invention is an organic chemical compound of the following formula:



in which formula E represents a single bond, a carbonyl group or one of the following groups:  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  
 30  $(-NR^{11}-)$ , in which n represents an integer from 1 to 2. The

substituents groups  $R^1$ - $R^{11}$  may be identical or different, independently being one of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

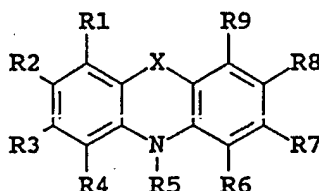
or in which specific formula two of the substituent groups  $R^5$  and  $R^6$  may together form a group -B-, in which B represents the groups:  $(-CH=N-)_n$ ,  $(-CH=CH-)$  or  $(-CHR^{14}-)$  in which groups n represents an integer of from 1 to 2 and  $R^{14}$  is a substituent group as defined above.

In particular embodiments, the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic



acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodiphenylamine, 4,4'-diaminodiphenylaminesulfate, 2,7-diaminofluorene, triphenylamine.

In another specific embodiment, the enhancing agent 5 may be described by the following formula:



in which formula X represents one of the following groups: (-O-), (-S-), (-NR<sup>15</sup>-), (-CHR<sup>15</sup>-), (>C=O), (-CH=CH-), (-CH=N-) and the substituent groups R<sup>1</sup>-R<sup>9</sup> and R<sup>15</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be substituted or substituted once or twice with a substituent group R<sup>10</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>;

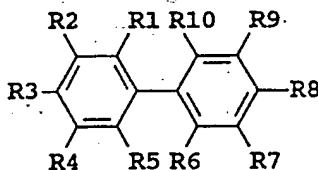
which substituent group R<sup>10</sup> represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals:

halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following the groups: (-CHR<sup>10</sup>-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub> or (-N=CR<sup>10</sup>-NR<sup>11</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>10</sup> is a substituent group as defined above and R<sup>11</sup> is defined as R<sup>10</sup>.

In particular embodiments, the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate or 10-ethyl-4-phenothiazine-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropylphenothiazine, methyl-10-phenothiazinepropionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)phenothiazine, chlorpromazine, 2-chloro-10-methylphenothiazine, 2-acetyl-10-methylphenothiazine, 4-carboxy-10-phenothiazine, 10-methylphenoxazine, 10-ethyl-phenoxazine, 10-phenoxazine-propionic acid or 4-carboxy-10-phenoxazine-propionic acid.

In another specific embodiment, enhancing agent is a biphenyl derivative of the following formula:



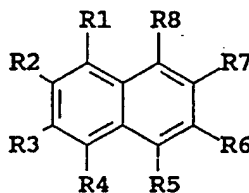
in which the substituent groups R<sup>1</sup>-R<sup>10</sup>, which may be identical or different, independently represents any of the

following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>11</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>11</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>11</sup>;

which substituent group R<sup>11</sup> represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

In particular embodiments, the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethylbenzidine, 4'-hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

In another specific embodiment, the enhancing agent is a naphthalene derivative of the following formula:



in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl,

carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following groups:  $(-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ ,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

In particular embodiments, the enhancing agent is 6-hydroxy-2-naphtoic acid, 6-bromo-2-naphtol, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, 1,6-dibromo-2-naphtol or 7-methoxy-2-naphtol.

The enhancing agent of the invention may be in free form or in the form of an addition salt.

#### Methods of Oxidizing a Substrate

In another aspect, the invention provides a method of oxidizing a substrate with a source of hydrogen peroxide in the presence of a peroxidase enzyme or a peroxidase acting compound, in the presence of an enhancing agent of the invention.

The enhancing agent of the invention may be present in free form or in the form of an addition salt.

The enhancing agent of the invention may be present in concentrations of from 0.01 to 500  $\mu$ M, more preferred 0.1 to 250  $\mu$ M, most preferred 1 to 100  $\mu$ M.

The source of hydrogen peroxide may be hydrogen peroxide or a hydrogen peroxide precursor for in situ production of hydrogen peroxide, e.g. percarbonate or perborate, or a hydrogen peroxide generating enzyme system, e.g. an oxidase and a substrate for the oxidase or an amino acid oxidase and a suitable amino acid, or a peroxycarboxylic acid or a salt thereof. Hydrogen peroxide may be added at the beginning or during the process, e.g. in an amount of 0.001-5 mM, particularly 0.01-1 mM.

### Peroxidases and Peroxidase Acting Compounds

The enzyme employed in the method of the invention may be any peroxidase enzyme comprised by the enzyme classification EC 1.11.1.7, or any fragment derived therefrom, exhibiting peroxidase activity, or synthetic or semisynthetic derivatives thereof (e.g. porphyrin ring systems or microperoxidases, cf. e.g. US Patent 4,077,768, EP Patent Application 537,381, International Patent Applications WO 91/05858 and WO 92/16634). Such enzymes are known from microbial, plant and animal origins.

Preferably, the peroxidase employed in the method of the invention is producible by plants (e.g. horseradish or soy bean peroxidase) or microorganisms such as fungi or bacteria. Some preferred fungi include strains belonging to the subdivision Deuteromycotina, class Hyphomycetes, e.g. Fusarium, Humicola, Trichoderma, Myrothecium, Verticillium, Arthromyces, Caldariomyces, Ulocladium, Embellisia, Cladosporium or Dreschlera, in particular Fusarium oxysporum (DSM 2672), Humicola insolens, Trichoderma resii, Myrothecium verrucana (IFO 6113), Verticillium alboatrum, Verticillium dahliae, Arthromyces ramosus (FERM P-7754), Caldariomyces fumago, Ulocladium chartarum, Embellisia allior Dreschlera halodes.

Other preferred fungi include strains belonging to the subdivision Basidiomycotina, class Basidiomycetes, e.g. Coprinus, Phanerochaete, Coriolus or Trametes, in particular Coprinus cinereus f. microsporus (IFO 8371), Coprinus macrorhizus, Phanerochaete chrysosporium (e.g. NA-12) or Trametes (previously called Polyporus), e.g. T. versicolor (e.g. PR4 28-A).

Further preferred fungi include strains belonging to the subdivision Zygomycotina, class Mycoraceae, e.g. Rhizopus or Mucor, in particular Mucor hiemalis.

Some preferred bacteria include strains of the order Actinomycetales, e.g. Streptomyces spheroides (ATTC 23965), Streptomyces thermoviolaceus (IFO 12382) or Streptoverticillium verticillium ssp. verticillium

Other preferred bacteria include Bacillus pumilus (ATCC 12905), Bacillus stearothermophilus, Rhodobacter sphaeroides, Rhodomonas palustri, Streptococcus lactis, Pseudomonas purrocinia (ATCC 15958) or Pseudomonas fluorescens (NRRL B-11).

5 Further preferred bacteria include strains belonging to Myxococcus, e.g. M. virescens.

Other potential sources of useful particular peroxidases are listed in Saunders B C, op. cit., pp. 41-43.

The peroxidase may furthermore be one which is  
10 producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said peroxidase as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the peroxidase, in a culture medium under  
15 conditions permitting the expression of the peroxidase and recovering the peroxidase from the culture.

Particularly, a recombinantly produced peroxidase is a peroxidase derived from a Coprinus sp., in particular C. macrorrhizus or C. cinereus according to WO 92/16634.

20 In the context of this invention, peroxidase acting compounds comprise peroxidase active fragments derived from cytochromes, hemoglobin or peroxidase enzymes, and synthetic or semisynthetic derivatives thereof, e.g. iron porphins, iron porphyrins, and iron phthalocyanine and derivatives thereof.

## 25 Industrial Applications

Due to their excellent performance at alkaline conditions, the enhancing agents of the invention, and hence the method for oxidizing a substrate with a peroxidase enzyme or a peroxidase acting compound in the presence of a source of  
30 hydrogen peroxide and in the presence of an enhancing of the invention, find various industrial applications

In a preferred embodiment, the method of the invention finds application for bleaching of dye in solutions.

In another embodiment, the method of the invention  
35 finds application for dye transfer inhibition, e.g. for

treatment of dyed textiles (cf. e.g. WO 92/18687) or during laundering (cf. e.g. WO 91/05839).

Accordingly, in a specific embodiment, the invention provides a method for inhibiting the transfer of a textile dye  
5 from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the method comprising treatment of the wash liquor with a peroxidase enzyme or a peroxidase acting compound in the presence of a source of hydrogen peroxide, and in the presence of an enhancing agent of  
10 the invention. The textile dye may be a synthetic dye such as an azo dye, or a natural or nature-identical dye.

In a third embodiment, the method of the invention finds application in bleaching of pulp for paper production. The use of a peroxidase together with hydrogen peroxide or a  
15 hydrogen peroxide precursor in bleaching of paper pulp has been described in e.g. SE 88/0673 and US 4,690,895.

Accordingly, the invention provides a method for bleaching of lignin-containing material, in particular bleaching of pulp for paper production, which method comprises  
20 treatment of the lignin or lignin containing material with a peroxidase enzyme or a peroxidase acting compound in the presence of a source of hydrogen peroxide and in the presence of an enhancing agent of the invention.

In a fourth embodiment, the method of the invention  
25 finds application for lignin modification, e.g. in particle board production. Binders for producing wood composites such as fibre boards and particle boards can be made from peroxidase treated lignin (cf. US 4,432,921).

Accordingly, the invention provides a method for  
30 enzymatic polymerization and/or modification of lignin or lignin containing material, which method comprises treatment of the lignin or lignin containing material with a peroxidase or a peroxidase acting compound in the presence of a source of hydrogen peroxide, and in the presence of an enhancing agent of  
35 the invention.

In a fifth embodiment, the method of the invention finds application in treatment of waste water e.g. waste water



from the chemical or pharmaceutical industry, from dye manufacturing, from dye-works, from the textile industry, or from pulp production (cf. e.g. US 4,623,465, or JP-A-2-31887).

In a more specific aspect, the invention provides a method for treatment of waste water from dye manufacturing, from dye-works, from textile industry, or from pulp manufacturing, the method comprising treatment of the waste water with a peroxidase or a peroxidase acting compound in the presence of a source of hydrogen peroxide and in the presence of an enhancing agent of the invention.

#### Detergent Compositions

Due to their excellent performance at alkaline conditions the enhancing agents of the invention are particularly well suited for implementation into detergent compositions, intended for performance in the range of pH 7-13, particularly the range of pH 8-12, preferably the range of pH 8-11.

According to the invention, the enhancing agent may be added as a component of a detergent composition. As such, it may be included in the detergent composition in the form of a detergent additive. The detergent composition as well as the detergent additive may additionally comprise one or more other enzymes conventionally used in detergents, such as proteases, lipases, amylases, oxidases, and cellulases.

In a specific aspect, the invention provides a detergent additive. The enzymes may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, i.e. a separated additive or a combined additive, can be formulated e.g. as granulates, liquids, slurries, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, slurries, or protected enzymes.

Dust free granulates may be produced, e.g. as disclosed in US 4,106,991 and US 4,661,452, and may optionally

be coated by methods known in the art. The detergent enzymes may be mixed before or after granulation.

Liquid enzyme preparations may, for instance, be stabilized using conventional stabilizing agents for the 5 enzymes, as described below.

Protected enzymes may be prepared according to the method disclosed in EP 238,216 A.

In another specific aspect, the invention provides a detergent composition capable of inhibiting the transfer of 10 textile dyes from dyed fabrics to other fabrics when said fabrics are washed together in a wash liquor, the detergent composition comprising an enzyme or compound exhibiting peroxidase activity, a source of hydrogen peroxide and an enhancing agent of the invention.

15 The enhancing agent of the invention may be included in the detergent composition as a part of a peroxidase system, comprising one or more peroxidase enzymes or peroxidase acting compounds, a source of hydrogen peroxide, and the enhancing agent of the invention.

20 The detergent composition of the invention may be in any convenient form, e.g. as powder, granules or liquid. A liquid detergent may be aqueous, typically containing up to 70% water and 0-20% organic solvent.

The detergent composition comprises one or more 25 surfactants each of which may be anionic, non-ionic, cationic or amphoteric. The detergent will usually contain 5-30% of anionic surfactant such as linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (AS), alcohol ethoxysulfate (AES) or soap. It may also contain 3-20% of non- 30 ionic surfactant such as nonylphenol ethoxylate or alcohol ethoxylate.

The detergent composition may additionally comprise one or more other enzymes, such as an amylase, lipase, cellulase or protease.

35 The detergent may contain 1-40% of a detergent builder such as zeolite, phosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid

(EDTA) or diethylenetriaminepentaacetic acid (DTPA), alkenylsuccinic anhydride, or silicate, or it may be unbuilt (i.e. essentially free of a detergent builder). It may also contain other conventional detergent ingredients, e.g. fabric conditioners, foam boosters, anti-corrosion agents, soil-suspending agents, sequestering agents, anti-soil redeposition agents, stabilizing agents for the enzyme(s), foam depressors, dyes, bactericides, optical brighteners or perfumes.

The pH (measured in aqueous detergent solution) will usually be neutral or alkaline, e.g. 7-11.

Particular forms of detergent composition within the scope of the invention include:

a) A detergent composition formulated as a detergent powder containing phosphate builder, anionic surfactant, nonionic surfactant, silicate, alkali to adjust to desired pH in use, and neutral inorganic salt.

b) A detergent composition formulated as a detergent powder containing zeolite builder, anionic surfactant, nonionic surfactant, acrylic or equivalent polymer, silicate, alkali to adjust to desired pH in use, and neutral inorganic salt.

c) A detergent composition formulated as an aqueous detergent liquid comprising anionic surfactant, nonionic surfactant, organic acid, alkali, with a pH in use adjusted to a value between 7 and 11.

d) A detergent composition formulated as a non-aqueous detergent liquid comprising a liquid nonionic surfactant consisting essentially of linear alkoxylated primary alcohol, phosphate builder, alkali, with a pH in use adjusted to a value between about 7 and 11.

e) A compact detergent composition formulated as a detergent powder in the form of a granulate having a bulk density of at least 600 g/l, containing anionic surfactant and nonionic surfactant, phosphate builder, silicate, and little or substantially no neutral inorganic salt.

f) A compact detergent composition formulated as a detergent powder in the form of a granulate having a bulk

density of at least 600 g/l, containing anionic surfactant and nonionic surfactant, zeolite builder, silicate, and little or substantially no neutral inorganic salt.

g) A detergent composition formulated as a detergent powder containing anionic surfactant, nonionic surfactant, acrylic polymer, fatty acid soap, carbonate, sulfate, clay particles, and silicate.

h) A liquid compact detergent comprising 5-65% by weight of surfactant, 0-50% by weight of builder and 0-30% by weight of electrolyte.

i) A compact granular detergent comprising linear alkylbenzenesulfonate, tallow alkyl sulfate,  $C_{14-15}$  alkyl sulfate,  $C_{14-15}$  alcohol 7 times ethoxylated, tallow alcohol 11 times ethoxylated, dispersant, silicone fluid, trisodium citrate, citric acid, zeolite, maleic acid/acrylic acid copolymer, diethylenetriaminepentakis(methylenephosphonic acid), cellulase, protease, lipase, amylase, sodium silicate, sodium sulfate, PVP, perborate and bleach activator.

j) A granular detergent comprising sodium linear  $C_{11-12}$  alkylbenzenesulfonate, sodium sulfate, zeolite A, sodium nitrilotriacetate, cellulase, PVP, tetraacetylenediamine, boric acid and perborate.

k) A liquid detergent comprising  $C_{12-14}$  alkenylsuccinic acid, citric acid, sodium  $C_{12-15}$  alkyl sulfate, sodium sulfate of  $C_{12-15}$  alcohol 2 times ethoxylated,  $C_{12-15}$  alcohol 7 times ethoxylated,  $C_{12-15}$  alcohol 5 times ethoxylated, diethylenetriaminepentakis(methylenephosphonic acid), oleic acid, ethanol, propanediol, protease, cellulase, PVP, suds suppressor, sodium hydroxide, perborate and bleach activator.

The following examples further illustrate the present invention, and they are not intended to be in any way limiting to the scope of the invention as claimed.

## EXAMPLE 1

Bleaching of Methyl Orange

Coprinus cinereus peroxidase (CiP) was obtained according to WO 92/16634, and purified to a single band on SDS-PAGE by the following method:

The crude peroxidase preparation was precipitated with 25% w/w ammoniumsulfate, and after centrifugation the precipitate was dissolved in 10 mM phosphate pH 7 (buffer A) and dialysed against the same buffer. The sample was applied onto a HighLoad Q-Sepharose column (Pharmacia, Sweden) equilibrated with buffer A, washed with buffer and eluted with a linear gradient of NaCl up to 0.5 M in the same buffer.

The main fraction containing peroxidase activity was collected, concentrated by ultrafiltration (with a membrane cut-off of 10kD) and dialysed against buffer A.

The concentration of CiP was determined by  $A_{404}$  using a molar absorption of  $109 \text{ mM}^{-1} \text{ cm}^{-1}$ .

Accelerated bleaching of Methyl Orange (Merck) catalysed by CiP and hydrogen peroxide in the presence of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS, supplied by Boehringer Mannheim) or para-hydroxybenzene sulfonate (pHBS, supplied by Sigma) is shown in Fig. 2. The following conditions were used:

10 nM CiP  
25  $\mu\text{M}$  Methyl Orange  
50  $\mu\text{M}$  ABTS or para-hydroxybenzene sulfonate  
20 or 200  $\mu\text{M}$  hydrogen peroxide  
50 mM Britton & Robinson buffer\*, pH 8.8  
30°C thermostat  
\* $\text{H}_3\text{PO}_4$ ,  $\text{CH}_3\text{CO}_2\text{H}$ ,  $\text{H}_3\text{BO}_3$ , all three components at a concentration of 50 mM

Reagents were mixed in a 1 cm cuvette, and the bleaching was started by addition of hydrogen peroxide. The bleaching was detected spectrophotometrically at 465 nm, which

is the absorption peak of this dye. Bleaching was followed with respect to time over a span of 10 min.

## EXAMPLE 2

### Bleaching of Methyl Orange

5 Accelerated bleaching of Methyl Orange (Merck) catalysed by a Coprinus cinereus peroxidase (CiP), obtained according to Example 1, and hydrogen peroxide in the presence of varying concentrations of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS, supplied by Boehringer Mannheim)  
10 is shown in Fig. 3. The following conditions were used:

10 nM CiP  
25  $\mu$ M Methyl Orange  
0, 1, 5 or 10  $\mu$ M ABTS  
200  $\mu$ M hydrogen peroxide  
15 50 mM Britton & Robinson buffer, pH 8.8  
30°C thermostat

Mixture, start and detection of the bleaching are as described in Example 1.

## EXAMPLE 3

### 20 Bleaching of Direct Blue 1

The initial bleaching of Direct Blue 1 (DB1) by a Coprinus cinereus peroxidase (CiP), obtained according to Example 1, using a selection of enhancers according to the invention was compared to the known enhancer, p-hydroxybenzene  
25 sulfonate, sodium salt (pHBS). Chemicals were obtained from Sigma-Aldrich, Janssen Chimica, Kodak, Tokyo Kasai Organic Chemicals, Daiichi Pure Chemicals Co. or Boehringer Mannheim, and some N-methylated derivatives of phenothiazine were made by methylation with methyl iodide as described by Cornel Bodea and  
30 Ioan Silberg in "Recent Advances in the Chemistry of Phenothiazines" (Advances in heterocyclic chemistry, 1968, Vol. 9,

pp. 321-460); B. Cardillo & G. Casnati in Tetrahedron, 1967, Vol. 23, p. 3771.

Due to low solubility some of the enhancers were dissolved in a small volume of ethanol before dilution in water.

The following conditions were used:

		Final concentration
	200 $\mu$ l 50 mM Britton-Robinson buffer,	
	pH 8.5 and 10.5, respectively,	10 mM
10	200 $\mu$ l DB1 ~ 3.0 Abs. Units (610 nm)	0.6 ( $A_{610nm}$ )
	200 $\mu$ l 50 nM CiP in water	10 nM
	200 $\mu$ l 50 $\mu$ M enhancer	10 $\mu$ M
	200 $\mu$ l 100 $\mu$ M $H_2O_2$	20 $\mu$ M

Reagents were mixed in a 1 cm thermostated cuvette at 30°C and the bleaching was started by addition of hydrogen peroxide.

The bleaching was detected spectrophotometrically at 610 nm, which is the absorption peak of DB1. After 5 sec. bleaching was followed for 4 minutes, and the initial bleaching rates (reduction in milli-absorbance units per minute, -  $\Delta mAbs/min$ , determined from the initial slope of the absorbance curve after 5 sec.) as well as the total bleaching within 4 minutes ( $100 \times (A_{610nm,start} - A_{610nm,4min}) / A_{610nm,start} \%$ ) were determined.

$A_{610nm,start}$  was determined by replacement of hydrogen peroxide with water.

From the results presented in Table 1, below, it appears that the enhancers of the invention are superior to the known enhancer, PHBS, in initial bleaching of DB1, especially at high pH values.

Determination of the bleaching after 4 minutes is carried out to verify that a reasonable total bleaching can be obtained, and data are due to possible hydrogen peroxide deficiency not directly comparable.

Table 1Bleaching of Direct Blue 1 at pH 8.5 and pH 10.5

Enhancer	Initial DB1 bleaching (-ΔmAbs/min)		DB1 bleaching in 4 min.	
	pH 8.5	pH 10.5	pH 8.5	pH 10.5
5				
ABTS	1044	197	86%	86%
10-Phenothiazine- propionic acid	1080	468	85%	88%
10 10-methyl- phenothiazine	1176	480	85%	87%
4'-hydroxy- 4-biphenyl- carboxylic acid	588	68	57%	39%
15 3,3',5,5'- tetramethyl- benzidine	960	266	63%	49%
4,4'- diaminostilbene-				
20 2,2'-disulfonic acid	222	61	58%	34%
4-amino-4'- methoxy-stilbene	132	72	46%	26%
1,5-diamino-				
25 naphthalene	240	90	37%	32%
6-hydroxy-2- naphthoic acid	486	52	46%	32%
10-ethyl-4-pheno- thiazinecarboxylic				
30 acid	1146	864	85%	89%
10-ethylpheno- thiazine	1098	624	83%	84%
10-propylpheno- thiazine	1068	299	80%	81%



Enhancer	Initial DB1 bleaching (-ΔmAbs/min)		DB1 bleaching in 4 min.	
	pH 8.5	pH 10.5	pH 8.5	pH 10.5
5 10-isopropylpheno- thiazine	681	59	83%	41%
methyl-10-pheno- thiazinepropionate	840	99	86%	67%
10-phenylpheno- 10 thiazine	498	40	81%	25%
10-allylpheno- thiazine	1170	183	86%	80%
N-hydroxysuccin- imide-10-pheno- 15 azinepropionic acid	1110	378	84%	75%
10-(3-(4-methyl-1- piperazinyl)propyl) phenothiazine	1092	237	87%	82%
10-(2-pyrrolidino- 20 ethyl)phenothia- zine	444	160	86%	80%
Chlorpromazine	222	26	61%	16%
10-methylpheno- zine	1464	792	79%	81%
25 6-amino-3-methyl-2- benzothiazolinone azine w. 3-methyl-2- benzothiazolinone	96	42	54%	29%
iminostilbene	186	56	35%	21%
30 2-(p-aminophenyl)- 6-methylbenzothia- zole-7-sulfonic acid	114	26	25%	11%
N-benzylidene- 4-biphenylamine	474	38	66%	28%
35 4,4'-diaminodiphe- nylaminesulfate	378	28	59%	8%

Enhancer	Initial DB1 bleaching (-ΔmAbs/min)		DB1 bleaching in 4 min.	
	pH 8.5	pH 10.5	pH 8.5	pH 10.5
5 5-amino-2-naphthalenesulfonic acid	816	63	71%	26%
1,6-dibromo-2-naphthol	222	58	70%	23%
7-methoxy-2-naphthol	480	84	46%	29%
10 4,4'-dihydroxybenzophenone	238	10	81%	4%
N-(4-(dimethylamino)benzylidene)-p-anisidine	294	24	49%	9%
15 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazine	378	26	66%	8%
20 2,7-diamino-2,7-difluorene, 2HCl	636	516	39%	49%
2-chloro-10-methylpheno-thiazine	225	15	86%	8%
2-acetyl-10-methylpheno-thiazine	270	25	88%	18%
PHBS	57	~0	42%	~0%
30 No enhancer	25	~0	18%	~0%

## EXAMPLE 4

Bleaching of Direct Blue 1 with ABTS

The initial bleaching of Direct Blue 1 (DB1) by a  
 35 Coprinus cinereus peroxidase (CiP), obtained according to  
 Example 1, using ABTS as accelerator, was compared to the best  
 of the hitherto known accelerators: 7-hydroxycoumarin (7HCm),

vanillin (VAN), and p-hydroxybenzene sulfonate (pHBS). The following conditions were used:

- 1 nM CiP or 100 nM CiP (at pH 10.5)
- 0, 10, 25, 50, or 75  $\mu$ M accelerator, respectively
- 5 50 mM Britton & Robinson buffer, pH 8.8 or 10.5, respectively
- 20  $\mu$ M hydrogen peroxide

Reagents were mixed in a 1 cm cuvette, and the bleaching was started by addition of hydrogen peroxide. The bleaching was detected spectrophotometrically at 610 nm, which is the absorption peak of this dye. Bleaching was followed for 10 minutes, and the bleaching rates ( $-\Delta mAbs/min$ ) were determined from the initial (linear) reduction in absorbance.

At pH 10.5 the bleaching using 100 nM CiP and ABTS as accelerator was so fast that bleaching was already completed before the cuvette could be placed in the spectrophotometer, the reason why the dosage of CiP at pH 10.5 was reduced to 1 nM when used in combination with ABTS, although a dosage near 100 nM CiP was necessary for all other (hitherto known) accelerators in order to see a significant reduction in absorbance.

The results of initial bleaching rate per minute have been illustrated in Figs. 4 and 5 as function of accelerator concentration.

#### EXAMPLE 5

##### 25 Enhanced Dye Transfer Inhibition by ABTS

A washing trial was carried out in a Terg-o-tometer to investigate the effect of ABTS on peroxidase based dye transfer inhibition. For a comparison, also the established enhancer pHBS was tested.

30 Clean white tracer test pieces (cotton, Style#400 from Testfabrics, Inc., USA; bleached, but unbrightened) were washed together with nylon test pieces dyed with the azo dye Acid Red 151 (C.I. 26900; available, e.g. from Aldrich Chemical

Co.). Reference test pieces were cut out of the same cotton cloth and washed in the absence of dyed fabric. The dye transfer in a given Terg-o-tometer pot was measured as the Hunter colour difference

5

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

between the tracer pieces in that pot and the above reference pieces, the Hunter L, a, and b values being evaluated from remission data obtained with an unfiltered daylight source on  
10 a Datacolor Elrephometer 2000.

The detergent solution for the washing trial was made up using 4.5 g/l of a commercially available European high-pH powder detergent containing no bleach and no optical brightener. The water used was tap water mixed with deminerali-  
15 zed water in the ratio 1:2; the mixture had a hardness equivalent to approx. 1.1 mM  $\text{Ca}^{2+}$ .

The detailed experimental conditions were:

Duration of wash:	15 min.
Terg-o-tometer agitation:	70 rotations/min.
20 Temperature:	35°C
pH:	Adjusted to 10.5 with NaOH prior to addition of peroxidase system
Textile load:	Approx. 6 g nylon dyed with acid Red 151 and 1 g white cotton per litre washing liquor
25 Peroxide source:	In all cases, 50 $\mu\text{M}$ $\text{H}_2\text{O}_2$ was present together with the peroxidase
30 Peroxidase:	<u>Coprinus cinereus</u> peroxidase obtained according to Example 1 at 5 nM

After washing, the test pieces were rinsed thoroughly in cold tap water and dried in the dark overnight, after which the remission measurements were performed.

Treatments with various concentrations of the two enhancers yielded the following results:

Hunter  $\Delta E$  with respect to  
white, washed fabric

---

	1 $\mu$ M ABTS	34.9
10	5 $\mu$ M ABTS	32.3
	20 $\mu$ M ABTS	23.7
	1 $\mu$ M PHBS	34.8
	5 $\mu$ M PHBS	34.5
	20 $\mu$ M PHBS	30.8

15 Differences of  $\geq 2$  units of Hunter  $\Delta E$  were statistically significant.

In both cases, the peroxidase system with 1  $\mu$ M enhancer provided no significant dye transfer inhibition (reference without peroxidase system not included here).  
20 However, as is seen that the ABTS system has an effect already at 5  $\mu$ M of enhancer, whereas the PHBS system does not; and at 20  $\mu$ M enhancer, the ABTS system has a much larger effect than the PHBS system.

#### EXAMPLE 6

##### 25 Bleaching of Direct Blue 1

A crude soy bean peroxidase (SBP) was purified by anion and cation chromatography followed by gelfiltration to a single protein on SDS-PAGE with an  $R_2$ -value ( $A_{404nm}/A_{280nm}$ ) of 2.2:

125 ml of crude SBP were adjusted to pH 7, diluted  
30 to 2.3 mS and filtered through 0.8  $\mu$  filter. The sample was applied to 300 ml of DEAE column equilibrated with 20 mM phosphate pH 7.0, and the peroxide was eluted with a 1 M NaCl

linear gradient in the same buffer. Fractions with peroxidase activity were pooled.

Pooled fractions from anion exchange chromatography (190 ml) were concentrated and washed by ultrafiltration (GR61PP) membrane from Dow, Denmark). pH was adjusted to 5.3 ionic strength to 2.3 mS in the sample before application to a 200 ml S-Sepharose column previously equilibrated with 50 mM acetate pH 5.3. The effluent containing the peroxidase activity was concentrated and washed by ultrafiltration to a final 10 volume of approx. 10 ml.

A 5 ml concentrated sample from cation exchange chromatography was applied to a 90 cm Sephacryl S-200 column equilibrated and eluted with 0.1 M acetate pH 6.1.

Fractions with peroxidase activity giving only one band on SDS-PAGE were pooled.

The bleaching rate of direct blue 1 (DB1) by the purified SBP was determined using a selection of enhancers according to the invention. The following conditions were used:

		Final concentration
20	200 $\mu$ l 50 mM Britton-Robinson buffer pH 6, 8 and 10, respectively	10 mM
	200 $\mu$ l DB1 ~ 3.0 Abs.Units (610 nm)	0.6 ( $A_{610nm}$ )
	200 $\mu$ l SBP with $A_{404nm} = 0.0005$ at pH 6 and 8 or with $A_{404nm} = 0.005$ at pH 10	0.0001 or 0.001 ( $A_{404nm}$ )
25	200 $\mu$ l 50 $\mu$ M enhancer	10 $\mu$ M
	200 $\mu$ l 100 $\mu$ M $H_2O_2$	20 $\mu$ M

Reagents were mixed in a thermostated cuvette at 30°C and the bleaching was started by addition of hydrogen peroxide.

The bleaching was detected and calculated as in Example 3.

From the results presented in Tables 2 and 3 below, it appears that by adding enhancers of the invention we can obtain a much faster bleaching of the dye compared to the 35 experiments without enhancer.

Table 2Bleaching of Direct Blue 1 with SBP in 4 Minutes

Enhancer	% DB1 bleaching in 4 min.		
	pH 6	pH 8	pH 10 10x[SBP]
5 No enhancer	0.7	<0.7	<0.7
10-Phenothiazine			
10 propionic acid	72	61	21
10-Ethyl-4-phenothiazine carboxylic acid	69	69	32
10-Methylphenothiazine	67	54	12
4'-Hydroxy-4-biphenyl-			
15 carboxylic acid	61	47	10
10-Methylphenoxazine	68	67	65
PHBS	<0.7	<0.7	<0.7

Table 3Initial Bleaching of Direct Blue 1 with SBP

Enhancer	(-ΔmAbs/min)	pH 8	pH 10 10x[SBP]
	pH 6		
5			
No enhancer	<1	<1	<1
10-Phenothiazine propionic acid	162	84	33
10 10-Ethyl-4-phenothiazine carboxylic acid	228	120	45
10-Methylphenothiazine	204	102	30
4'-Hydroxy-4-biphenyl- carboxylic acid	237	132	9
15 10-Methylphenoxazine	258	180	89
<hr/>			
pHBS	<1	<1	<1

**EXAMPLE 7**

20 Myxococcus virescens, DSM 8593, was inoculated on "5  
CY" agar plates with the following composition:

	Casitone	3 g
	Yeast extract	1 g
	CaCl <sub>2</sub> ·2H <sub>2</sub> O	1 g
25	Agar	15 g
	Water ad 1000 ml, pH 7.2	

and incubated for 2 weeks at 26°C.

The agar was cut into pieces and transferred  
aseptically to 5 shake flasks with 100 ml "MD-1M"-medium in  
30 each flask.

The flasks were incubated on a rotary shaker at 250  
rpm, 26°C, for 5 days. The combined culture from the 5 flasks



was used for inoculating 50 flasks with 100 ml "MD-1M"-medium in each, using a 10 ml inoculum per flask.

The 50 flasks were incubated on a rotary shaker at 250 rpm, 26°C, for 3 days. The peroxidase activity as described above was measured in the combined culture from the 50 flasks, result: 0.2 PODU/ml.

After separation of the solid material by centrifugation the peroxidase was concentrated by ultrafiltration using a 10 kDa cut off membrane. The ultrafiltrated preparation had an activity of 2.9 PODU/ml.

The ultrafiltrated preparation described above was used in a dye bleaching experiment with Direct Blue 1 (DB1) at pH 10.5 (Fig. 6), with and without an enhancer of the invention, using the following conditions:

15                    100  $\mu$ M H<sub>2</sub>O<sub>2</sub>  
                    25 mM Borate pH 10.5  
                    0.5 PODU/ml Myxococcus virescens peroxidase  
                    0 or 10  $\mu$ M 10-phenothiazinepropionic acid, respectively, room temperature  
20                    the initial concentration of DB1 was adjusted to give an OD<sub>610nm</sub> = 0.6.

Reagents were mixed in a 1 cm cuvette, and the bleaching was started by addition of hydrogenperoxide. The bleaching was detected spectrophotometrically at 610 nm for 120  
25 seconds.

Fig. 6 shows that the effect of the enhancer is very pronounced. It also shows that the absorbance is reduced to at least half the initial value after 30 sec. in the experiment with 10  $\mu$ M 10-propionic acid phenothiazine.

30

#### EXAMPLE 8

##### Bleaching of Direct Blue 1

Horse radish peroxidase type VI (HRPC) was obtained from Sigma (8P-8375).

The bleaching rate of direct blue 1 (DB1) by HRPC was determined using a selection of enhancers according to the invention. The following conditions were used:

		Final concentration
5	200 $\mu$ l 50 mM Britton-Robinson buffer pH 8 and 10, respectively	10 mM
	200 $\mu$ l DB1 ~ 3.0 Abs.Units (610 nm)	0.6 ( $A_{610nm}$ )
	200 $\mu$ l HRPC with $A_{404nm}=0.005$	0.001 ( $A_{404nm}$ )
	200 $\mu$ l 50 $\mu$ M enhancer	10 $\mu$ M
10	200 $\mu$ l 100 $\mu$ M $H_2O_2$	20 $\mu$ M

Reagents were mixed in a thermostated cuvette at 30°C, and the bleaching was started by addition of hydrogen peroxide.

The bleaching was detected and calculated as in  
15 Example 3.

From the results presented in Table 4 below, it appears that by adding enhancers of the invention we can obtain a much faster bleaching of the dye compared to the experiment without enhancer.

Table 4Initial Bleaching of Direct Blue 1 with HRPC

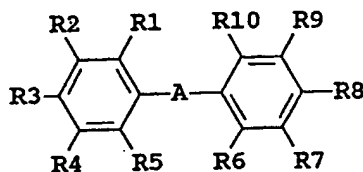
Enhancer	-ΔmAbs/min	
	pH 8	pH 10
5		
No enhancer	<1	<1
10-Phenothiazine propionic acid	1061	62
10 10-Ethyl-4-phenothiazine carboxylic acid	1038	68
10-Methylphenothiazine	1164	83
10-Methylphenoxazine	1188	99
15 pHBS	<2	<2

MICROORGANISMS	
Optional Sheet in connection with the microorganism referred to on page <u>38</u> , line <u>20</u> of the description	
A. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution *	
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH	
Address of depositary institution (including postal code and country) *	
Mascheroder Weg 1b, D-3300 Braunschweig, Federal Republic of Germany	
Date of deposit *	Accession Number *
30 September 1993	DSM 8593
B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet <input type="checkbox"/>	
<p>In respect of those designations in which a European patent is sought, a sample of the deposited micro-organism will be made available only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC) until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or is deemed to be withdrawn.</p>	
C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (If the indications are not for all designated States)	
<p>D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable)</p> <p>The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")</p>	
<p>E. <input type="checkbox"/> This sheet was received with the international application when filed (to be checked by the receiving Office)</p> <p style="text-align: center;"><i>Ernst J. Steinhilber</i> (Authorized Officer)</p> <p><input type="checkbox"/> The date of receipt (from the applicant) by the International Bureau is</p> <p style="text-align: center;">was _____ (Authorized Officer)</p>	

## CLAIMS

1. A method of oxidizing a substrate with a peroxidase enzyme or a peroxidase acting compound in the presence of a source of hydrogen peroxide, characterized by the presence of an enhancing agent being an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

2. A method according to claim 1 in which the enhancing agent is described by the general formula I:



in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-NR^{11}-)_n$ ,  $(-CR^{11}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{13}-NR^{15}-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)_n$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)_n$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)_n$ ,  $(-N=N-)_n$ , in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or phosphor, which carbon, phosphor and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, sulfandiyl, nitro, amino, phenyl,  $C_1$ - $C_4$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and

which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, and aryl- $C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

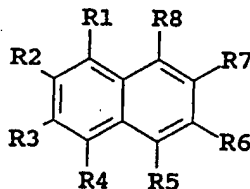
which substituent group  $R^{14}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1-C_5$ -alkyl, and  $C_1-C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula  $R^5$  and  $R^6$  may together form a group -B-, in which B represents a single bond, the group  $(-CH=CH-)_n$ , or the group  $(-CH=N-)_n$ , in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{14}$  as defined above;

or in which general formula two of the substituent groups  $R^1-R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to 4, and in which groups  $R^{11}$  is a substituent group as defined above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when  $n$  in the above formula being 1, may together form a group -D-, in which D represents the groups:  $(-CHR^{11}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=CR^{15}-)$ ,  $(-CR^{11}=N-)$ ,  $(-N=CR^{11}-)$ ,  $(-O-)$ ,  $(>C=O)$  or  $(-S-)$ , and in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

3. A method according to claim 1, in which the enhancing agent is described by the general formula II:



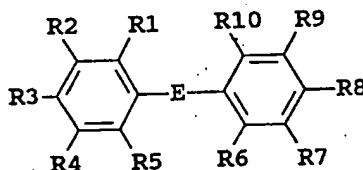
in which the substituent groups  $R^1-R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, aryl- $C_1-C_5$ -alkyl; which carbamoyl, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, and aryl- $C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, nitro, amino, phenyl,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with

hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>3</sub>-alkyl, and C<sub>1</sub>-C<sub>3</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

4. A method according to either of claims 1-2, in which the enhancing agent is described by the following formula:



in which formula E represents a single bond, a carbonyl group or one of the following groups: (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub> or (-NR<sup>11</sup>-), in which n represents an integer from 1 to 2. The substituents groups R<sup>1</sup>-R<sup>11</sup> may be identical or different, independently being one of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>4</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino



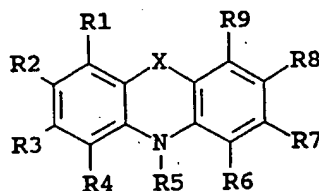
groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, 5 carbonyl- $C_1-C_5$ -alkyl, and aryl- $C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the 10 following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof. nitro, amino, phenyl,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may 15 furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1-C_3$ -alkyl,  $C_1-C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and 20 sulfamoyl; and which  $C_1-C_5$ -alkyl, and  $C_1-C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and 25 esters and salts hereof, and sulfamoyl;

or in which specific formula two of the substituent groups  $R^5$  and  $R^6$  may together form a group -B-, in which B represents the groups:  $(-CH=N-)_n$ ,  $(-CH=CH-)$  or  $(-CHR^{14}-)$  in which groups n represents an integer of from 1 to 2 and  $R^{14}$  is a 30 substituent group as defined above.

5. A method according to claim 4, in which the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodiphenylamine, 4,4'-diaminodiphenylaminesulfate, 2,7-diaminofluorene, triphenylamine.

6. A method according to either of claims 1-2, in which the enhancing agent is described by the following formula:



in which formula X represents one of the following  
 5 groups: (-O-), (-S-), (-NR<sup>15</sup>-), (-CHR<sup>15</sup>-), (>C=O), (-CH=CH-), (-CH=N-), (-N=N-) and the substituent groups R<sup>1</sup>-R<sup>9</sup> and R<sup>15</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and  
 10 esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with  
 15 a substituent group R<sup>10</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>; and which C<sub>1</sub>-C<sub>14</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be  
 20 unsubstituted or substituted with one or more substituent groups R<sup>10</sup>;

which substituent group R<sup>10</sup> represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts  
 25 hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters

and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

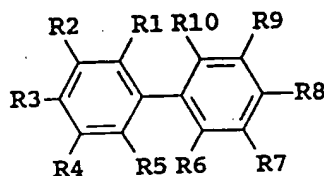
or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following the groups: (-CHR<sup>10</sup>-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub> or (-N=CR<sup>10</sup>-NR<sup>11</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>10</sup> is a substituent group as defined above and R<sup>11</sup> is defined as R<sup>10</sup>.

7. A method according to claim 6, in which X in the formula is (-O-) or (-S-) and R<sup>5</sup> in the formula is an alkyl group which may be further substituted.

8. A method according to either of claims 6-7, in which the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate or 10-ethyl-4-phenothiazine-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropylphenothiazine, methyl-10-phenothiazinepropionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)-phenothiazine, chlorpromazine, 2-chloro-10-methylphenothiazine, 2-acetyl-10-methylphenothiazine, 4-carboxy-10-phenothiazine-propionic acid, 10-methylphenoxazine, 10-ethylphenoxazine, 10-

phenoxazine-propionic acid or 4-carboxy-10-phenoxazine-propionic acid.

9. A method according to either of claims 1-2, in which the enhancing agent is a biphenyl derivative of the following formula:



in which the substituent groups  $R^1$ - $R^{10}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{11}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ;

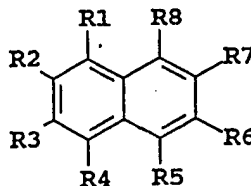
which substituent group  $R^{11}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters

and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may further-  
 5 more be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may  
 10 ed, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

10. A method according to claim 9, in which the  
 15 enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethylbenzidine, 4'-hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

11. A method according to either of claims 1-2, in which the enhancing agent is 2,2'-azino-bis(3-ethylbenzo-  
 20 thiazoline-6-sulfonate), 6-amino-3-methyl-2-benzothiazolinone azine with 3-methyl-2-benzothiazolinone, 2-(p-aminophenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethylamino)-benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazone.

25 12. A method according to either of claims 1 and 3, in which the enhancing agent is a naphthalene derivative of the following formula:



in which the substituent groups  $R^1-R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, aryl- $C_1-C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, and aryl- $C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1-C_3$ -alkyl,  $C_1-C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1-C_5$ -alkyl, and  $C_1-C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group  $-B-$ , in which B represents any of the following groups:  $(-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ ,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n repre-

sents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

13. A method according to claim 12, in which the enhancing agent is 6-hydroxy-2-naphtoic acid, 6-bromo-2-naphtol, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diamino-naphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, 1,6-dibromo-2-naphtol or 7-methoxy-2-naphtol.

10 14. A method according to any of claims 1-13, in which said method is a method for bleaching of dye in solutions.

15 15. A method according to any of claims 1-13, in which said method is a method for inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor.

16. A method according to any of claims 1-13, in which said method is a method for bleaching of lignin-containing material, in particular bleaching of pulp for paper  
20 production.

17. A method according to any of claims 1-13, in which said method is a method for enzymatic polymerization and/or modification of lignin or lignin containing material.

18. A method according to any of claims 1-13, in  
25 which said method is a method for treatment of waste water, in particular waste water from the pharmaceutical or chemical industry, e.g. waste water from dye manufacturing, from textile industry, or from pulp manufacturing.

19. A method according to any of claims 14-18, in which the enhancing agent is added at the beginning of, or during the process.

20. A method according to any of claims 14-19, in which the amount of enhancing agent is in the range of from 0.01-500  $\mu\text{M}$ , more preferred 0.1-250  $\mu\text{M}$ , most preferred 1-100  $\mu\text{M}$ .

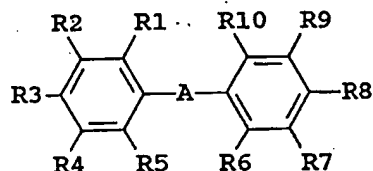
21. A method according to any of claims 14-20, in which the source of hydrogen peroxide is hydrogen peroxide or a hydrogen peroxide precursor, e.g. percarbonate or perborate, or a hydrogen peroxide generating enzyme system, e.g. an oxidase and its substrate, or a peroxycarboxylic acid or a salt thereof.

22. A method according to any of claims 14-21, in which the peroxidase enzyme is horseradish peroxidase or soy bean peroxidase or a peroxidase enzyme derived from Coprinus, e.g. C. cinereus or C. macrorrhizus, or from Bacillus, e.g. B. pumilus, or Myxococcus, e.g. M. virescens.

23. A detergent additive capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent additive comprising a peroxidase enzyme or a compound exhibiting peroxidase activity, a source of hydrogen peroxide, characterized by the presence of an enhancing agent being an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

24. A detergent additive according to claim 23, in which the enhancing agent is described by the general formula I:





in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{13}-NR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ , in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be

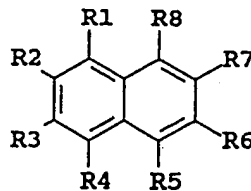
unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>3</sub>-alkyl, and C<sub>1</sub>-C<sub>3</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula R<sup>5</sup> and R<sup>6</sup> may together form a group -B-, in which B represents a single bond, the group (-CH=CH-)<sub>n</sub>, or the group (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group R<sup>14</sup> as defined above;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>10</sup> may together form a group -C-, in which C represents any of the following groups: (-CHR<sup>11</sup>-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, in which groups n represents an integer of from 2 to 4, and in which groups R<sup>11</sup> is a substituent group as defined above;

or in which general formula R<sup>5</sup> and R<sup>12</sup>, and/or R<sup>6</sup> and R<sup>13</sup>, when n in the above formula being 1, may together form a group -D-, in which D represents the groups: (-CHR<sup>11</sup>-), (-NR<sup>11</sup>-), (-CR<sup>11</sup>=CR<sup>15</sup>-), (-CR<sup>11</sup>=N-), (-N=CR<sup>11</sup>-), (-O-), (>C=O) or (-S-), and in which groups R<sup>11</sup> and R<sup>15</sup> are substituent groups as defined above.

25. A detergent additive according to claim 23, in which the enhancing agent is described by the general formula II:



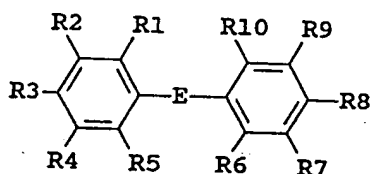
in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl,

carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following groups:  $(-N=N-)_n$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ ,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

26. A detergent additive according to either of claims 23-24, in which the enhancing agent is described by the following formula:



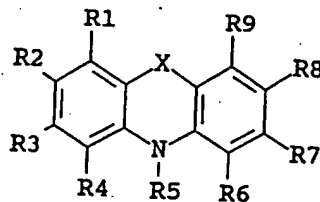
in which formula E represents a single bond, a carbonyl group or one of the following groups:  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-NR^{11}-)$ , in which n represents an integer from 1 to 2. The substituents groups  $R^1$ - $R^{11}$  may be identical or different, independently being one of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1-C_3$ -alkyl,  $C_1-C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals:  
10 halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1-C_5$ -alkyl, and  $C_1-C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any  
15 of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which specific formula two of the substituent groups  $R^5$  and  $R^6$  may together form a group -B-, in which B  
20 represents the groups:  $(-CH=N-)_n$ ,  $(-CH=CH-)$  or  $(-CHR^{14}-)$  in which groups n represents an integer of from 1 to 2 and  $R^{14}$  is a substituent group as defined above.

27. A detergent additive according to claim 26, in which the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-  
25 diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodiphenylamine, 4,4'-diaminodiphenylaminesulfate, 2,7-diaminofluorene, triphenylamine.

28. A detergent additive according to either of  
30 claims 23-24, in which the enhancing agent is described by the following formula:



in which formula X represents one of the following groups: (-O-), (-S-), (-NR<sup>15</sup>-), (-CHR<sup>15</sup>-), (>C=O), (-CH=CH-), (-CH=N-) and the substituent groups R<sup>1</sup>-R<sup>9</sup> and R<sup>15</sup>, which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>4</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R<sup>10</sup>; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>; and which C<sub>1</sub>-C<sub>4</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy, carbonyl-C<sub>1</sub>-C<sub>5</sub>-alkyl, and aryl-C<sub>1</sub>-C<sub>5</sub>-alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R<sup>10</sup>;

which substituent group R<sup>10</sup> represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and

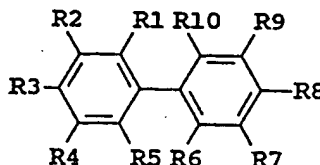
sulfamoyl; and which C<sub>1</sub>-C<sub>3</sub>-alkyl, and C<sub>1</sub>-C<sub>3</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following the groups: (-CHR<sup>10</sup>-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub> or (-N=CR<sup>10</sup>-NR<sup>11</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>10</sup> is a substituent group as defined above and R<sup>11</sup> is defined as R<sup>10</sup>.

29. A detergent additive according to claim 28, in which X in the formula is (-O-) or (-S-) and R<sup>5</sup> in the formula is an alkyl group which may be further substituted.

30. A detergent additive according to either of claims 28-29, in which the enhancing agent 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate or 10-ethyl-4-phenothiazine-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-isopropylphenothiazine, methyl-10-phenothiazinepropionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)-phenothiazine, chlorpromazine, 2-chloro-10-methylphenothiazine, 2-acetyl-10-methylphenothiazine or 10-methylphenoxazine.

31. A detergent additive according to either of claims 23-24, in which the enhancing agent is a biphenyl derivative of the following formula:



in which the substituent groups  $R^1$ - $R^{10}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{11}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ;

which substituent group  $R^{11}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

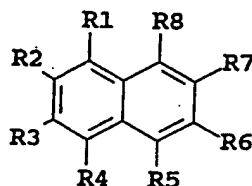
32. A detergent additive according to claim 31, in which the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethylbenzidine, 4'-



hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

33. A detergent additive according to either of claims 23-24, in which the enhancing agent is 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate), 6-amino-3-methyl-2-benzothiazolinone azine with 3-methyl-2-benzothiazolinone, 2-(p-aminophenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethylamino)benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazone.

34. A detergent additive according to either of claims 23 and 25, in which the enhancing agent is a naphthalene derivative of the following formula:



in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals:

10 halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any

15 of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following groups:  $(-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ ,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n represents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

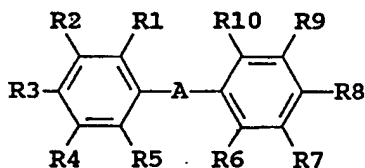
35. A detergent additive according to claim 34, in which the enhancing agent is 6-hydroxy-2-naphtic acid, 6-bromo-2-naphtol, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, 1,6-dibromo-2-naphtol or 7-methoxy-2-

30 naphtol.

36. A detergent additive according to any of claims 23-35, provided in the form of a granulate, preferably a non-dusting granulate, a liquid, in particular a stabilized liquid, a slurry, or a protected enzyme.

37. A detergent composition capable of inhibiting the transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, the detergent composition comprising a peroxidase enzyme or a compound exhibiting peroxidase activity, a source of hydrogen peroxide, characterized by the presence of an enhancing agent being an organic chemical compound consisting of at least two aromatic rings, of which aromatic rings at least one is substituted with one or more of the following atoms: nitrogen, oxygen, and sulfur; and which aromatic rings may furthermore be fused rings.

38. A detergent composition according to claim 37, in which the enhancing agent is described by the general formula I:



in which general formula A represents a single bond, or one of the following groups:  $(-CR^{11}=CR^{15}-)_n$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=N-)_n$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{13}-NR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-N=CR^{15}-)$ ,  $(-NR^{11}-CR^{12}=N-)_n$ ,  $(-CR^{12}=N-NR^{11}-)$ ,  $(-NR^{11}-CR^{12}=CR^{13}-)$ , in which groups n represents an integer of from 1 to 6; or A represents carbon, carbonyl, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{11}$ ;

and in which general formula the substituent groups  $R^1$ - $R^{13}$  and  $R^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino

groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, and aryl- $C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

which substituent group  $R^{14}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperazinyl, pyrrolidino,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1-C_5$ -alkyl, and  $C_1-C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

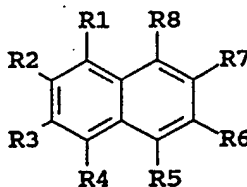
or in which general formula  $R^5$  and  $R^6$  may together form a group -B-, in which B represents a single bond, the group  $(-CH=CH-)_n$ , or the group  $(-CH=N-)_n$ , in which groups n represents an integer of from 1 to 6; or B represents carbon, nitrogen, sulfur, oxygen, selenium, or tellurium, which carbon and nitrogen may be unsubstituted or substituted with a substituent group  $R^{14}$  as defined above;

or in which general formula two of the substituent groups  $R^1-R^{10}$  may together form a group -C-, in which C represents any of the following groups:  $(-CHR^{11}-N=N-)_n$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ , in which groups n represents an integer of from 2 to

4, and in which groups  $R^{11}$  is a substituent group as defined above;

or in which general formula  $R^5$  and  $R^{12}$ , and/or  $R^6$  and  $R^{13}$ , when  $n$  in the above formula being 1, may together form a group -D-, in which D represents the groups:  $(-CHR^{11}-)$ ,  $(-NR^{11}-)$ ,  $(-CR^{11}=CR^{15}-)$ ,  $(-CR^{11}=N-)$ ,  $(-N=CR^{11}-)$ ,  $(-O-)$ ,  $(>C=O)$  or  $(-S-)$ , and in which groups  $R^{11}$  and  $R^{15}$  are substituent groups as defined above.

39. A detergent composition according to claim 38,  
in which the enhancing agent is described by the general formula II:



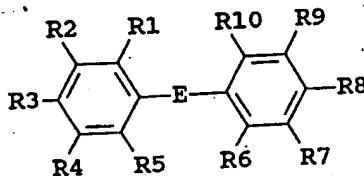
in which the substituent groups  $R^1$ - $R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and

esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may  
 5 furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and  
 10 sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and  
 15 esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups R<sup>1</sup>-R<sup>8</sup> may together form a group -B-, in which B represents any of the following groups: (-N=N-), (-CH=CH-)<sub>n</sub>, (-CH=N-)<sub>n</sub>, (-N=CR<sup>9</sup>-NR<sup>10</sup>-) or (-N=N-CR<sup>9</sup>-), in which groups n represents an integer of from 1 to 3, R<sup>9</sup> is a substituent group as  
 20 defined above and R<sup>10</sup> is defined as R<sup>9</sup>.

40. A detergent composition according to either of claims 37-38, in which the enhancing agent is described by the following formula:



in which formula E represents a single bond, a carbonyl group or one of the following groups:  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-NR^{11}-)$ , in which n represents an integer from 1 to 2. The substituents groups  $R^1$ - $R^{11}$  may be identical or different, independently being one of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{14}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ; and which  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{14}$ ;

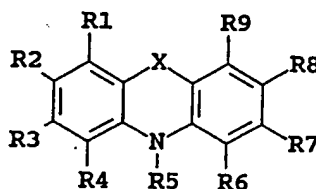
which substituent group  $R^{14}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_3$ -alkyl,  $C_1$ - $C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts here f, and sulfamoyl;

or in which specific formula two of the substituent groups  $R^5$  and  $R^6$  may together form a group -B-, in which B

represents the groups:  $(-\text{CH}=\text{N}-)_n$ ,  $(-\text{CH}=\text{CH}-)$  or  $(-\text{CHR}^{14}-)$  in which groups n represents an integer of from 1 to 2 and  $\text{R}^{14}$  is a substituent group as defined above.

41. A detergent composition according to claim 40, in which the enhancing agent is 4-amino-4'-methoxystilbene, 4,4'-diaminostilbene-2,2'-disulfonic acid, iminostilbene, 4,4'-dihydroxybenzophenone, N-benzylidene-4-biphenylamine, 4,4'-diaminodiphenylamine, 4,4'-diaminodiphenylaminesulfate, 2,7-diaminofluorene, triphenylamine.

42. A detergent composition according to either of claims 37-38, in which the enhancing agent is described by the following formula:



in which formula X represents one of the following groups:  $(-\text{O}-)$ ,  $(-\text{S}-)$ ,  $(-\text{NR}^{15}-)$ ,  $(-\text{CHR}^{15}-)$ ,  $(>\text{C}=\text{O})$ ,  $(-\text{CH}=\text{CH}-)$ ,  $(-\text{CH}=\text{N}-)$  and the substituent groups  $\text{R}^1$ - $\text{R}^9$  and  $\text{R}^{15}$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $\text{C}_1$ - $\text{C}_{14}$ -alkyl,  $\text{C}_1$ - $\text{C}_5$ -alkoxy, carbonyl- $\text{C}_1$ - $\text{C}_5$ -alkyl, aryl- $\text{C}_1$ - $\text{C}_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $\text{R}^{10}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $\text{R}^{10}$ ; and which  $\text{C}_1$ - $\text{C}_{14}$ -alkyl,  $\text{C}_1$ - $\text{C}_5$ -alkoxy, carbonyl- $\text{C}_1$ - $\text{C}_5$ -alkyl, and aryl- $\text{C}_1$ - $\text{C}_5$ -



alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{10}$ ;

which substituent group  $R^{10}$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl, aminoalkyl, piperidino, piperaziny, pyrrolidino,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1$ - $C_5$ -alkyl,  $C_1$ - $C_5$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1$ - $C_5$ -alkyl, and  $C_1$ - $C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

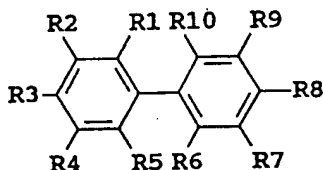
or in which general formula two of the substituent groups  $R^1$ - $R^8$  may together form a group -B-, in which B represents any of the following the groups:  $(-CHR^{10}-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$  or  $(-N=CR^{10}-NR^{11}-)$ , in which groups n represents an integer of from 1 to 3,  $R^{10}$  is a substituent group as defined above and  $R^{11}$  is defined as  $R^{10}$ .

43. A detergent composition according to claim 42, in which X in the formula is (-O-) or (-S-) and  $R^5$  in the formula is an alkyl group which may be further substituted.

44. A method according to either of claims 42-43, in which the enhancing agent is 10-methylphenothiazine, 10-phenothiazine-propionic acid, N-hydroxysuccinimide-10-phenothiazine-propionate or 10-ethyl-4-phenothiazine-carboxylic acid, 10-ethylphenothiazine, 10-propylphenothiazine, 10-

isopropylphenothiazine, methyl-10-phenothiazinepropionate, 10-phenylphenothiazine, 10-allylphenothiazine, 10-(3-(4-methyl-1-piperazinyl)propyl)phenothiazine, 10-(2-pyrrolidinoethyl)-phenothiazine, chlorpromazine, 2-chloro-10-methylphenothiazine,  
 5 2-acetyl-10-methylphenothiazine or 10-methylphenoxazine.

45. A detergent composition according to either of 37-38, in which the enhancing agent is a biphenyl derivative of the following formula:



in which the substituent groups  $R^1$ - $R^{10}$ , which may be  
 10 identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1$ - $C_{14}$ -alkyl,  $C_1$ -  
 15  $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, aryl- $C_1$ - $C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^{11}$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ; and which  
 20  $C_1$ - $C_{14}$ -alkyl,  $C_1$ - $C_5$ -alkoxy, carbonyl- $C_1$ - $C_5$ -alkyl, and aryl- $C_1$ - $C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^{11}$ ;

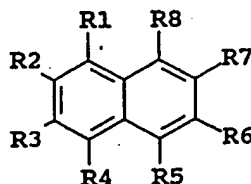
which substituent group  $R^{11}$  represents any of the  
 25 following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters

and salts thereof, nitro, amino, phenyl, C<sub>1</sub>-C<sub>5</sub>-alkyl, C<sub>1</sub>-C<sub>5</sub>-alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy, C<sub>1</sub>-C<sub>3</sub>-alkyl, C<sub>1</sub>-C<sub>3</sub>-alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which C<sub>1</sub>-C<sub>5</sub>-alkyl, and C<sub>1</sub>-C<sub>5</sub>-alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl.

46. A detergent composition according to claim 45, in which the enhancing agent is benzidine, 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, 3,3',5,5'-tetramethylbenzidine, 4'-hydroxy-4-biphenylcarboxylic acid, or 4,4'-dihydroxybiphenylene.

47. A detergent composition according to either of claims 37-38, in which the enhancing agent is 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate), 6-amino-3-methyl-2-benzothiazolinone azine with 3-methyl-2-benzothiazolinone, 2-(p-aminophenyl)-6-methylbenzothiazole-7-sulfonic acid, N-(4-(dimethylamino)benzylidene)-p-anisidine, 3-methyl-2-benzothiazolinone(4-(dimethylamino)benzylidene)hydrazone.

48. A detergent composition according to either of claims 37 and 39, in which the enhancing agent is a naphthalene derivative of the following formula:



in which the substituent groups  $R^1-R^8$ , which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, aryl- $C_1-C_5$ -alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group  $R^9$ ; and which phenyl may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ; and which  $C_1-C_{14}$ -alkyl,  $C_1-C_5$ -alkoxy, carbonyl- $C_1-C_5$ -alkyl, and aryl- $C_1-C_5$ -alkyl groups may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups  $R^9$ ;

which substituent group  $R^9$  represents any of the following radicals: halogen, hydroxy, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, phospho, phosphono, phosphonooxy and esters and salts thereof, nitro, amino, phenyl,  $C_1-C_5$ -alkyl,  $C_1-C_5$ -alkoxy; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy,  $C_1-C_3$ -alkyl,  $C_1-C_3$ -alkoxy; and which phenyl may furthermore be substituted with one or more of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl; and which  $C_1-C_5$ -alkyl, and  $C_1-C_5$ -alkoxy groups may furthermore be saturated or unsaturated, branched or unbranched, and may furthermore be substituted once or twice with any of the following radicals: halogen, hydroxy, amino, formyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, and sulfamoyl;

or in which general formula two of the substituent groups  $R^1-R^8$  may together form a group  $-B-$ , in which B represents any of the following groups:  $(-N=N-)$ ,  $(-CH=CH-)_n$ ,  $(-CH=N-)_n$ ,  $(-N=CR^9-NR^{10}-)$  or  $(-N=N-CR^9-)$ , in which groups n repre-

sents an integer of from 1 to 3,  $R^9$  is a substituent group as defined above and  $R^{10}$  is defined as  $R^9$ .

49. A detergent composition according to claim 48, in which the enhancing agent is 6-hydroxy-2-naphtoic acid, 6-bromo-2-naphtol, 7-methoxy-2-naphtol, 7-amino-2-naphthalene sulfonic acid, 5-amino-2-naphthalene sulfonic acid, 1,5-diaminonaphthalene, 7-hydroxy-1,2-naphthimidazole, 5-amino-2-naphthalenesulfonic acid, 1,6-dibromo-2-naphtol or 7-methoxy-2-naphtol.

50. A detergent composition according to any of claims 37-49, which further comprises one or more other enzymes, in particular a protease, a lipase, an amylase, a cellulase, and an oxidase.

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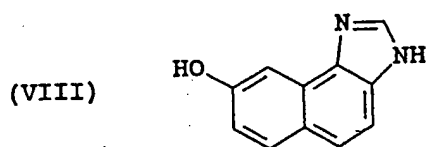
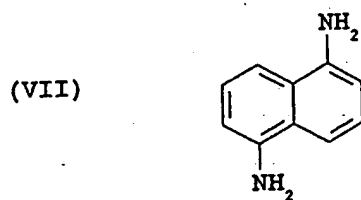
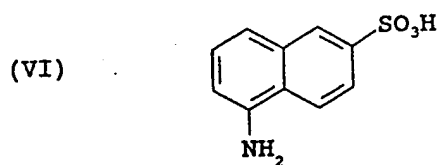
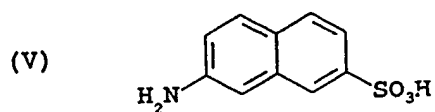
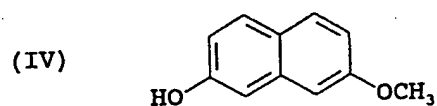
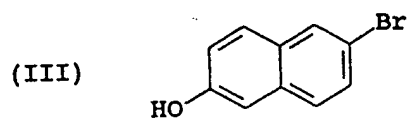
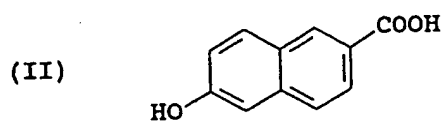
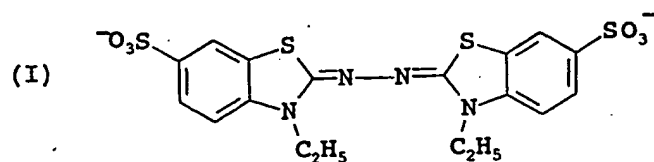
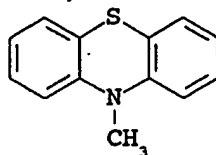


Fig. 1A

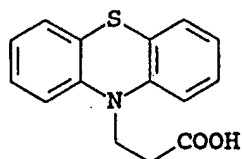
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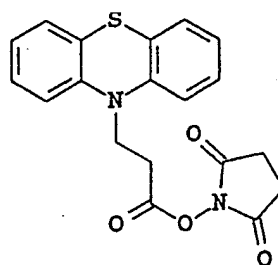
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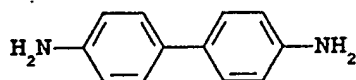
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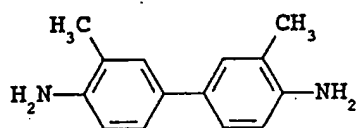
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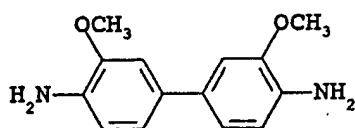
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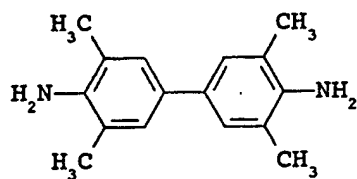
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(XIV)



(XV)



(XVI)

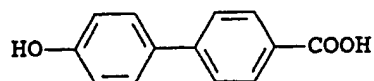


Fig. 1B

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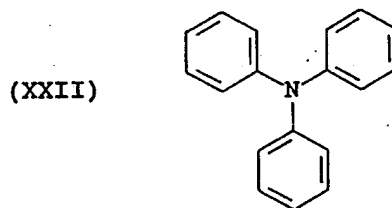
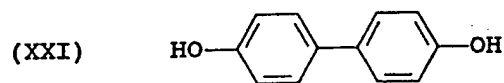
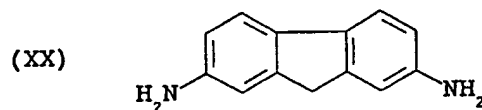
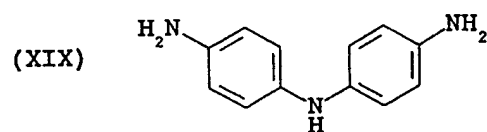
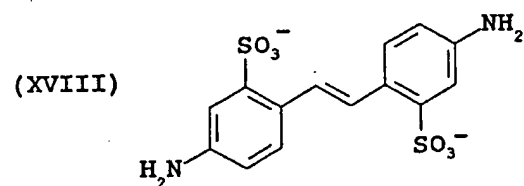
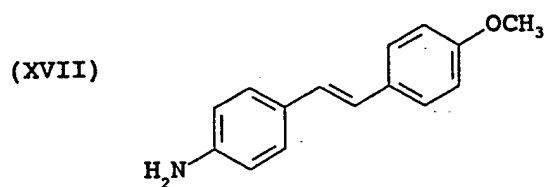


Fig. 1C  
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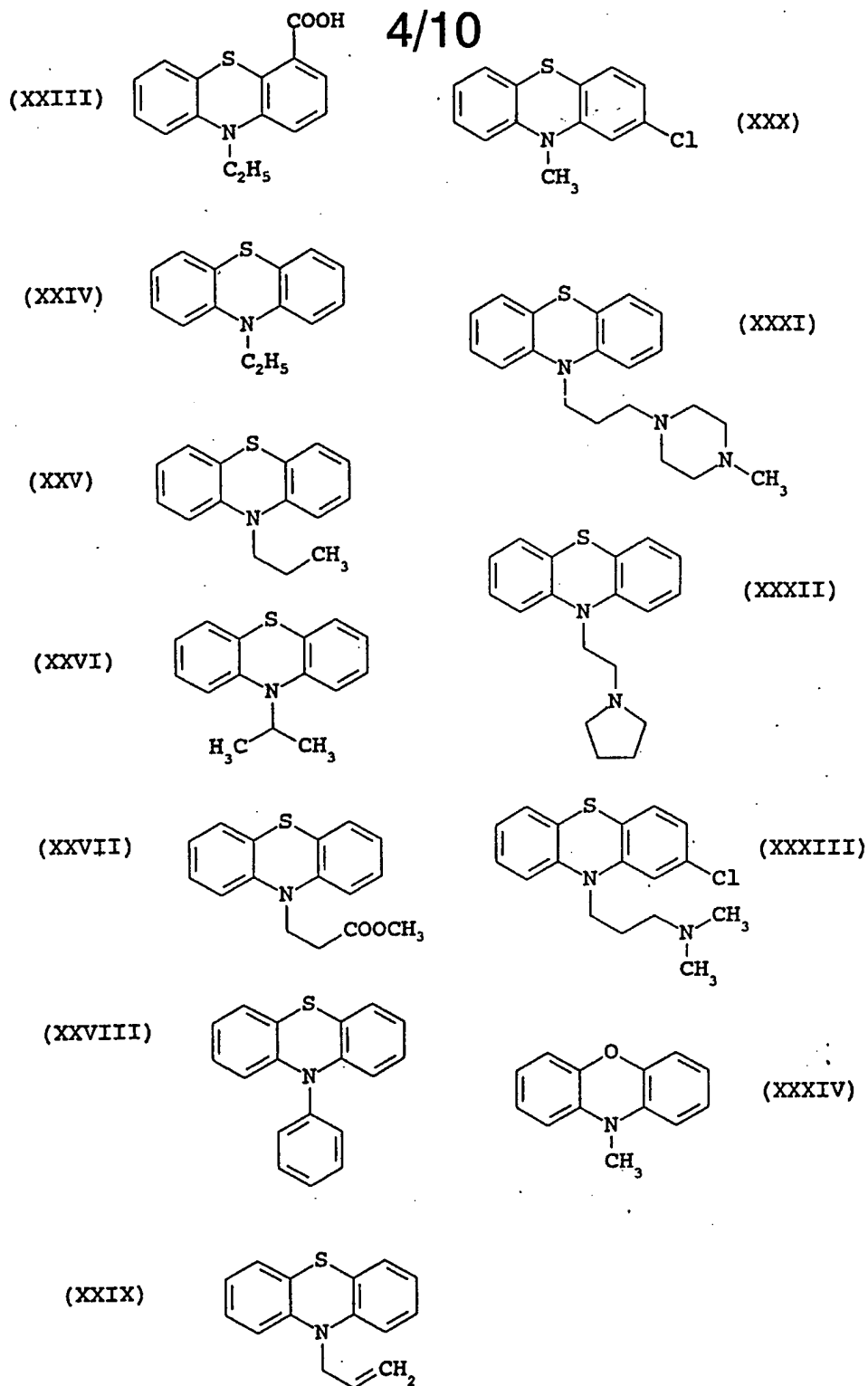


Fig. 1D  
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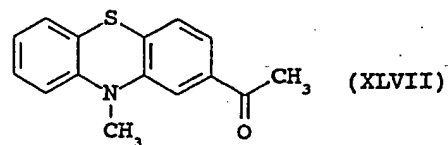
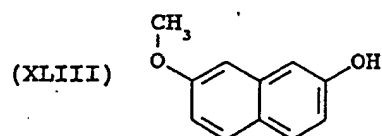
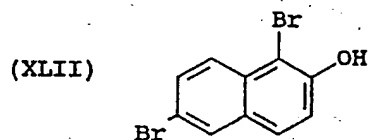
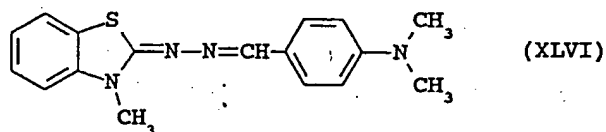
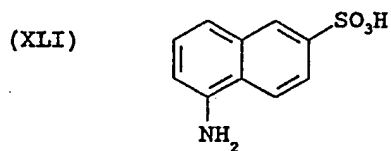
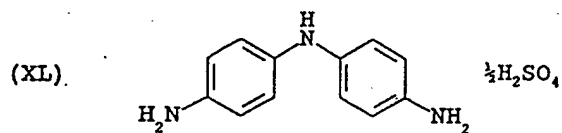
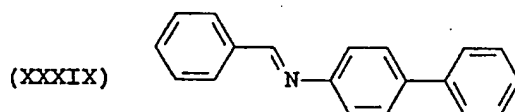
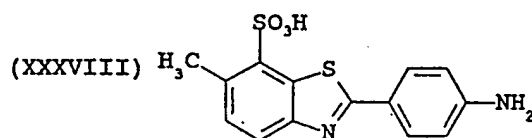
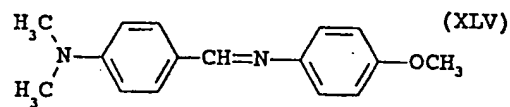
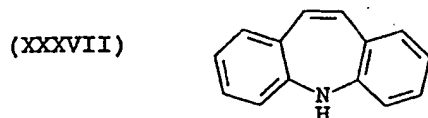
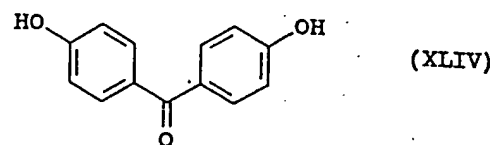
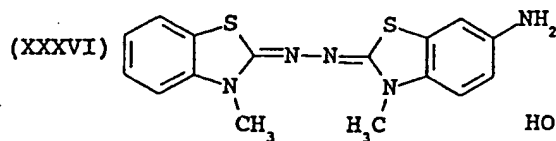


Fig. 1E

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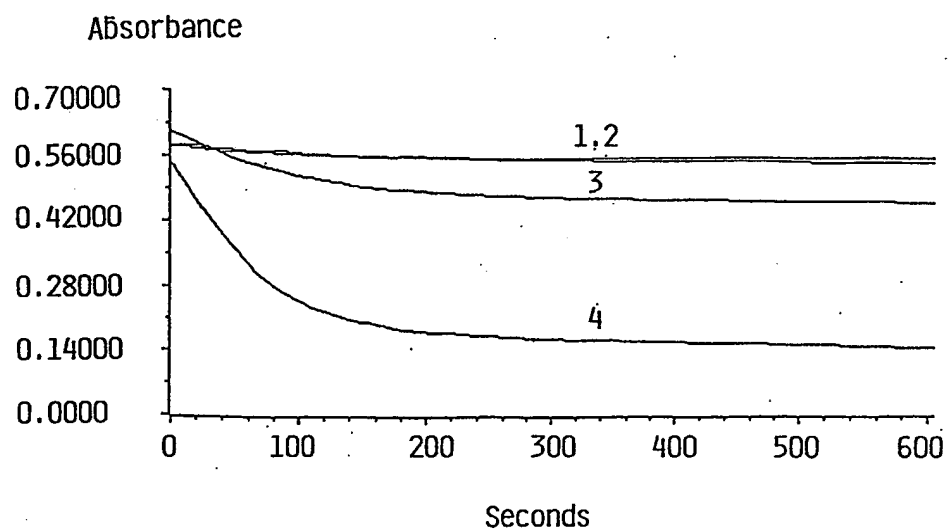


Fig. 2

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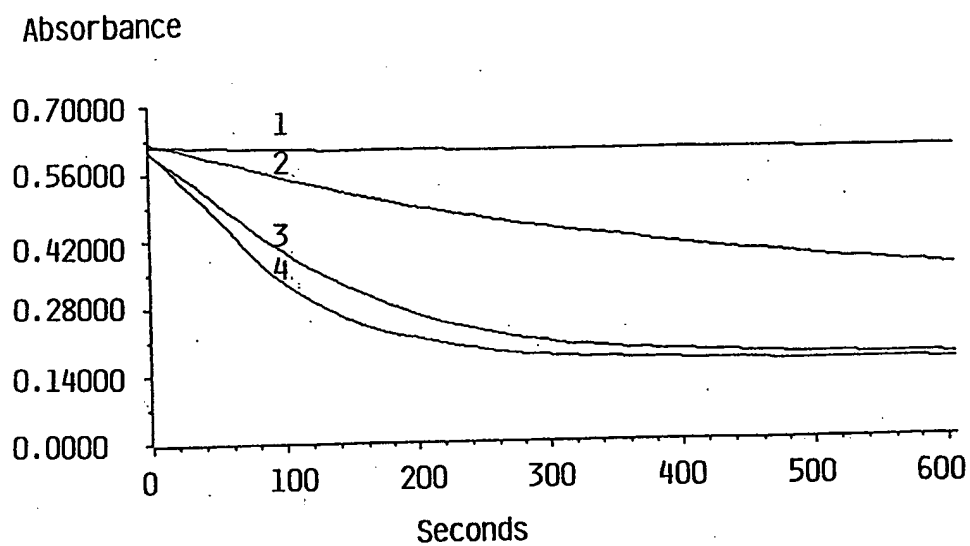


Fig. 3  
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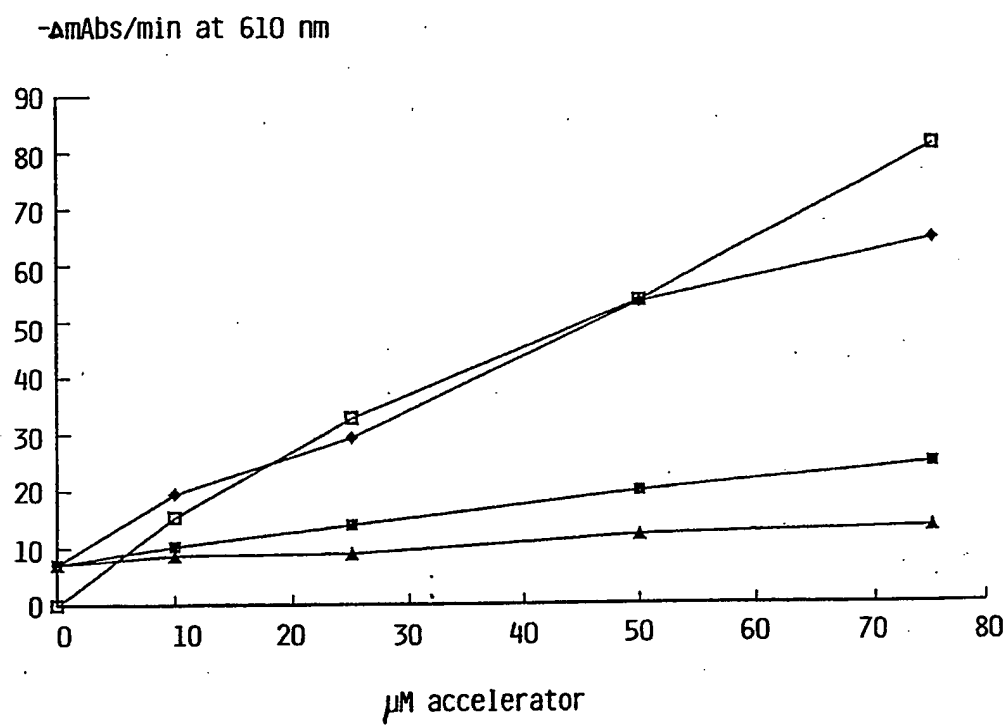


Fig. 4

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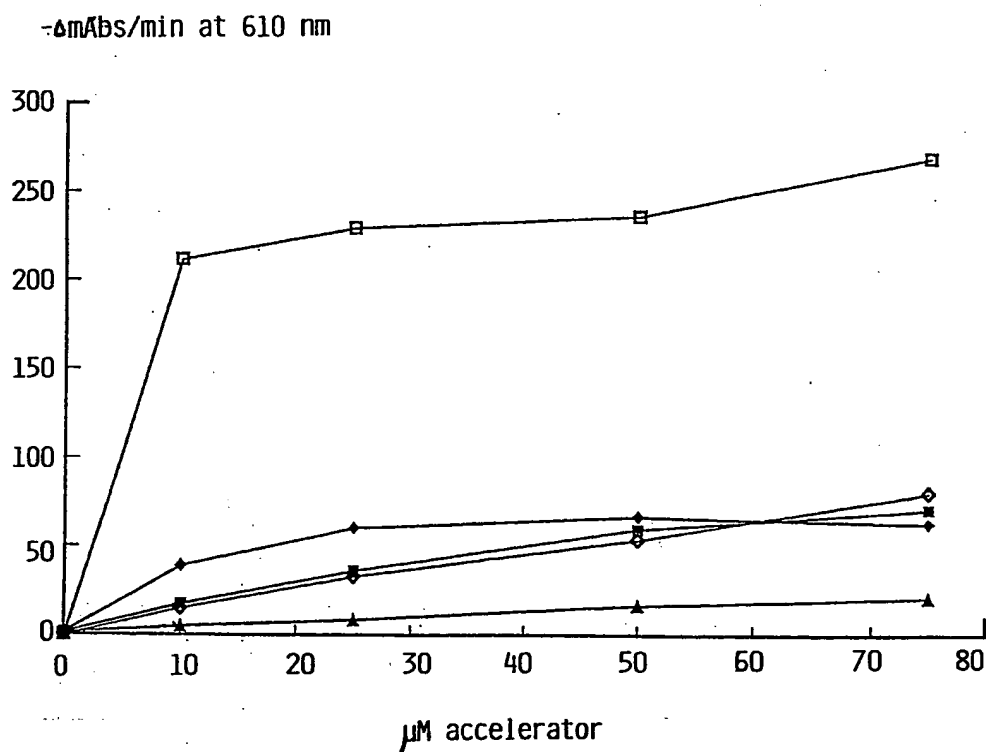


Fig. 5

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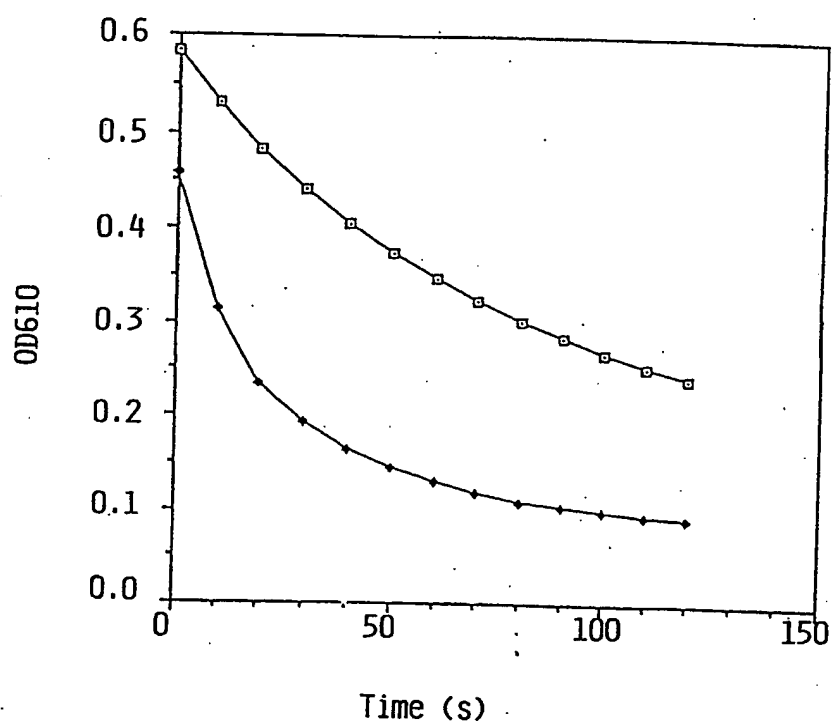


Fig. 6

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 93/00395

## A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C12N 9/08, C11D 3/386, D06L 3/02, D06P 5/00  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C12N, C11D, C07C, C07D, D06L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, WPI, WPIL, CLAIMS, CHEMICAL ABSTRACT

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, A2, 0361470 (FUJIREBIO INC.), 4 April 1990 (04.04.90), page 3, line 27; page 4, line 5, claims	1,6-8
Y	--	14-23,28-30, 36-37,42-44, 50
X	Dialog Information Services, File 5, BIOSIS, Dialog accession no. 5895700/5, BIOSIS accession no. 84028265, CHAKRABORTY R. et al: "Effect of reserpine on peroxidase activity in submaxillary gland", & INDIAN J BIOCHEM BIOPHYS 24 (2). 1987. 81-86	1
A	--	23,36,37,50

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&amp;" document member of the same patent family

Date of the actual completion of the international search

29 April 1994

Date of mailing of the international search report

05 -05- 1994

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 93/00395

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, File 5, BIOSIS, Dialog accession no. 5261765/5, BIOSIS accession no. 81029072, SCHRAMM H. et al: "Differential regulation of hepatic glutathione transferase and glutathione peroxidase activities in the rat", & BIOCHEM PHAR- MACOL 34 (20). 1985. 3735-3740	1-6,9-13
Y	--	14-27,31-36, 37-42,45-50
X	Dialog Information Services, File 5, BIOSIS, Dialog accession no. 7096898/5, BIOSIS accession no. 88019643, SANCHEZ O. J. et al: "Relation of cell wall peroxidase activity with growth in epicotyls of cicer-arietinum effects of calmodulin inhi- bitors", PHYSIOL PLANT 75 (2). 1989. 275-279	1,6-8
Y	--	14-17,19,21, 23,28-30, 36-37,42-44, 50
Y	WO, A1, 9218683 (NOVO NORDISK A/S), 29 October 1992 (29.10.92)	14,19-22
Y	WO, A1, 9218687 (NOVO NORDISK A/S), 29 October 1992 (29.10.92)	15,19-22
Y	US, A, 4690895 (R. L. FARRELL), 1 Sept 1987 (01.09.87), the claims	16,19-22
Y	US, A, 4432921 (A. HAARS ET AL), 21 February 1984 (21.02.84), column 2, line 41 - column 3, line 36, the claims	17,19-22

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 93/00395

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4623465 (A. M. KLIBANOV), 18 November 1986 (18.11.86) --	18-22
Y	WO, A1, 9105839 (NOVO NORDISK A/S), 2 May 1991 (02.05.91), see the claims --	23-50
A	DD, A, 147368 (PORTSMANN BÄRBEL ET AL), 1 April 1981 (01.04.81) --	1-50
A	US, A, 3893803 (E. T. KAISER), 8 July 1975 (08.07.75) --	1-2, 4-5, 9-11, 14-24, 26-27, 31-33
A	US, A, 4318984 (T. A. MAGERS ET AL), 9 March 1982 (09.03.82)	1-2, 4-5, 9-11, 23-24, 26, 31-33, 36-38, 40-41
A	----- --	45-47, 50

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK 93/00395

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See the attached sheet!

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 93/00395

The subjects included in the expression in claim 1 "at least two aromatic rings" are so different from each other that no technical relationships or interaction are seen to be present so as to form a single general invention.

The application contains the following inventions or groups of inventions:

Group I: claim 1 (partly), 2,4,5 (partly), 9-11, 14, 22, 23 (partly), 24, 26-27, 31-33, 36-37 (partly), 38, 40-41, 45-47, 50 (partly).

The claims include the compounds which are represented with formula I. These are used as an enhancer of the activity of peroxidase.

Group II: claim 1 (partly), 3, 12-13, 23 (partly), 25, 34-35, 36-37 (partly), 39, 48-49, 50 (partly).

the claims include the compounds which are represented with formula II. These are used as an enhancer of the activity of peroxidase.

Group III: claim 1 (partly), 6-8, 23 (partly), 28-30, 36-37 (partly), 42-44, 50 (partly).

The claims include the compounds which are represented with the formula shown in claim 6 (in application filed 23rd of December 1993).

The searching of the claims has been done essentially on the compounds which are represented in the examples.

INTERNATIONAL SEARCH REPORT  
Information on patent family members

26/02/94

International application No.  
PCT/DK 93/00395

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0361470	04/04/90	DE-D- 68911633 JP-A- 2174694 US-A- 5171668	00/00/00 06/07/90 15/12/92
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US-A- 4318984	09/03/82	AU-B- 528783 AU-A- 6281380 CA-A- 1143633 EP-A,B- 0029155 SE-T3- 0029155	12/05/83 21/05/81 29/03/83 27/05/81

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